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ORIGINAL ARTICLE

An Anti-CD3 Antibody, Teplizumab, in Relatives at Risk for Type 1 Diabetes

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ABSTRACT

BACKGROUND
Type 1 diabetes is a chronic autoimmune disease that leads to destruction of insulin-producing beta cells and dependence on exogenous insulin for survival. Some interventions have delayed the loss of insulin production in patients with type 1 diabetes, but interventions that might affect clinical progression before diagnosis are needed.

METHODS
We conducted a phase 2, randomized, placebo-controlled, double-blind trial of teplizumab (an Fc receptor–nonbinding anti-CD3 monoclonal antibody) involving relatives of patients with type 1 diabetes who did not have diabetes but were at high risk for development of clinical disease. Patients were randomly assigned to a single 14-day course of teplizumab or placebo, and follow-up for progression to clinical type 1 diabetes was performed with the use of oral glucose-tolerance tests at 6-month intervals.

RESULTS
A total of 76 participants (55 [72%] of whom were ≤18 years of age) underwent randomization — 44 to the teplizumab group and 32 to the placebo group. The median time to the diagnosis of type 1 diabetes was 48.4 months in the teplizumab group and 24.4 months in the placebo group; the disease was diagnosed in 19 (43%) of the participants who received teplizumab and in 23 (72%) of those who received placebo. The hazard ratio for the diagnosis of type 1 diabetes (teplizumab vs. placebo) was 0.41 (95% confidence interval, 0.22 to 0.78; P=0.006 by adjusted Cox proportional-hazards model). The annualized rates of diagnosis of diabetes were 14.9% per year in the teplizumab group and 35.9% per year in the placebo group. There were expected adverse events of rash and transient lymphopenia. TIGIT+KLRG1+CD8+ T cells were more common in the teplizumab group than in the placebo group. Among the participants who were HLA-DR3–negative, HLA-DR4–positive, or anti–zinc transporter 8 antibody–negative, fewer participants in the teplizumab group than in the placebo group had diabetes diagnosed.

CONCLUSIONS
Teplizumab delayed progression to clinical type 1 diabetes in high-risk participants. (Funded by the National Institutes of Health and others; ClinicalTrials.gov number, NCT01030861.)

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*A complete list of investigators in the Type 1 Diabetes TrialNet Study Group is provided in the Supplementary Appendix, available at NEJM.org.

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Type 1 diabetes is caused by the autoimmune destruction of insulin-producing beta cells in the islets of Langerhans, which leads to dependence on exogenous insulin for survival. Approximately 1 million to 1.5 million Americans have type 1 diabetes, which is one of the most common diseases of childhood (second-most-common after asthma).[1] Despite improvements in care, the desired glycemic targets are not achieved in most patients with type 1 diabetes,[2] and an increased risk of complications and death persists. Two studies involving Scottish men and women noted the loss of 14.2 and 17.7 life-years, respectively, among those in whom the condition was diagnosed before the age of 10 years and of 11 and 13 life-years, respectively, among those in whom it was diagnosed before the age of 20 years.[3,4]

In genetically susceptible persons, type 1 diabetes progresses through asymptomatic stages before the development of overt hyperglycemia. These stages are characterized by the appearance of autoantibodies (stage 1) and then dysglycemia (stage 2). In stage 2, metabolic responses to a glucose load are impaired, but other metabolic indexes — for example, the level of glycosylated hemoglobin — remain normal, and insulin treatment is not needed.[5] These immunologic and metabolic features can identify persons at high risk for development of clinical disease; overt hyperglycemia, once it develops, requires insulin treatment.

Several immune interventions, when studied in patients with recent-onset clinical type 1 diabetes, have been reported to delay the decline in beta-cell function.[6] One promising type of therapy appears to be Fc receptor–nonbinding anti-CD3 monoclonal antibodies, such as teplizumab; multiple studies involving patients with type 1 diabetes have shown that teplizumab treatment reduces the loss of beta-cell function, even as long as 7 years after diagnosis.[7-11] The drug modifies CD8+ T lymphocytes, which are thought to be the important effector cells that kill beta cells.[12,13]

Whether interventions at stage 1 or 2 might alter the progression to clinical type 1 diabetes has been unclear. We therefore tested whether teplizumab treatment would prevent or delay the onset of clinical type 1 diabetes in high-risk persons.

Methods

Trial Participants

Participants were identified through the TrialNet Natural History Study.[14] The trial was conducted from July 2011 through November 2018 at sites in the United States, Canada, Australia, and Germany (Fig. S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org). The full protocol is available at NEJM.org. Institutional-review-board approval was obtained at each participating site (see the Supplementary Appendix for a full listing). The participants, their parents, or both provided written informed consent or assent before trial entry.

Eligible participants were nondiabetic relatives of patients with type 1 diabetes and were at least 8 years of age at the time of randomization and at high risk for development of clinical diabetes. Participants also had to have had two or more diabetes-related autoantibodies detected in two samples obtained within 6 months before randomization. In addition, participants had to have had evidence of dysglycemia during an oral glucose-tolerance test, with dysglycemia defined as a fasting glucose level of 110 to 125 mg per deciliter (6.1 to 6.9 mmol per liter), a 2-hour postprandial plasma glucose level of at least 140 mg per deciliter (7.8 mmol per liter) and less than 200 mg per deciliter (11.1 mmol per liter), or an intervening postprandial glucose level at 30, 60, or 90 minutes of greater than 200 mg per deciliter on two occasions, within 52 days before enrollment. The protocol was amended in 2014 to allow enrollment of participants younger than 18 years of age who had a single abnormal oral glucose-tolerance test result, because the rates of type 1 diabetes progression were similar with or without a confirmatory oral glucose-tolerance test in this age group. In eight participants (five in the teplizumab group and three in the placebo group), the second pretreatment oral glucose-tolerance test was performed on the first day of administration of teplizumab or placebo. Persons with other clinically important medical histories, abnormal laboratory chemical values, or abnormal blood counts were excluded.

Trial Design and Intervention

Participants were randomly assigned in a 1:1 ratio to receive either teplizumab or placebo. Randomization was stratified according to TrialNet site, age (<18 years or ≥18 years), and second oral glucose-tolerance test result before treatment (impaired tolerance, normal tolerance, or diabetes). The treatment-group assignments were double-masked. Participants received a 14-day outpatient course of teplizumab or saline to be administered intravenously in a clinical research center. Teplizumab was given at a dose of 51 μg per square meter of body-surface area on day 0, a dose of 103 μg per square meter on day 1, a dose of 207 μg per square meter on day 2, and a dose of 413 μg per square meter on day 3, followed by a dose of 826 μg per square meter on each of days 4 through 13, as described previously.[7,10]

END POINTS AND ASSESSMENTS

The primary end point was the elapsed time from randomization to the clinical diagnosis of diabetes, determined with the use of criteria from the American Diabetes Association.[15] Scheduled oral glucose-tolerance tests were performed 3 months and 6 months after the infusions and every 6 months thereafter. Random screening glucose levels were evaluated at 3-month intervals, and an oral glucose-tolerance test was performed if the random glucose level was higher than 200 mg per deciliter (11.1 mmol per liter) in association with standardized symptoms of diabetes.

Oral glucose-tolerance test results that indicated diabetes were then sequentially confirmed, and the date of diagnosis was identified as the time of the first of the two diagnostic tests.[16] Outcomes were reviewed by the TrialNet Eligibility and Events Committee, the members of which were unaware of the treatment-group assignments.

TRIAL OVERSIGHT

The trial was developed and conducted by Type 1 Diabetes TrialNet, which is funded by the National Institutes of Health and the Juvenile Diabetes Research Foundation. MacroGenics was the holder of the investigational new drug application at the start of the trial. Currently, Provention Bio holds the application, and employees of Provention Bio reviewed the manuscript before submission.

The trial coordination, laboratory tests, and data management were conducted centrally, with the exception of complete blood count and differential and routine chemical analyses, which were performed at the infusion sites. Flow cytometry was performed centrally (Table S1 in the Supplementary Appendix). TrialNet investigators designed the trial. Members of the TrialNet Coordinating Center, including two of the authors, gathered and analyzed the data and vouch for the accuracy and completeness of the data and for the fidelity of the trial to the protocol. An independent medical monitor (who was unaware of the treatment-group assignments) reviewed all accruing safety data. MacroGenics provided teplizumab and matching placebo but was not involved in the conduct of the trial or in data analysis. Representatives from the sponsoring institute of the National Institutes of Health (National Institute of Diabetes and Digestive and Kidney Diseases) participated in the design and conduct of the trial; interpretation of the data; preparation, review, and approval of the manuscript for submission; and the decision to submit the manuscript for publication. The sponsor did not have the right or ability to veto submission for publication.

STATISTICAL ANALYSIS

The cumulative incidence of diabetes diagnosis within each group over time after randomization was estimated in a Kaplan–Meier analysis with the "diabetes-free" survival function.[17] The difference between the treatment groups in the 6-month-interval cumulative-incidence functions was estimated as the hazard ratio, and hypotheses were evaluated with the use of a likelihood-ratio test; both analyses were based on the Cox proportional-hazards model.[18]

Because of slower-than-expected rates of enrollment, the original protocol (which called for the enrollment of 144 participants) was revised to detect a 60% (previously 50%) lower risk in the teplizumab group than in the placebo group (i.e., a hazard ratio of 0.4) with 80% statistical power at an alpha level of 0.025 (one-sided). This update set the goal of enrolling at least 71 participants and following them until 40 participants had received a diagnosis of type 1 diabetes.[19]

Data on safety and efficacy were evaluated twice yearly by an independent data and safety monitoring board. An interim analysis was conducted when 18 (of 40) cases of type 1 diabetes had been observed, and a formal comparison was presented to the data and safety monitoring board.

Table 1. Baseline Characteristics of the Participants.*

Characteristic	Teplizumab (N=44)	Placebo (N=32)
Age — yr		
Median (IQR)	14 (12–22)	13 (11–16)
Range	8.5–49.5	8.6–45.0
Age <18 yr — no. (%)	29 (66)	26 (81)
Male sex — %	57	53
Relationship to person with type 1 diabetes — no. (%)		
Sibling†	28 (64)	16 (50)
Offspring	6 (14)	6 (19)
Parent	6 (14)	3 (9)
Sibling and another first-degree relative	2 (5)	3 (9)
Second-degree relative	2 (5)	3 (9)
Third-degree relative or further removed	0	1 (3)
Autoantibodies — no. of participants positive (%)‡		
Anti-GAD65, harmonized	40 (91)	28 (88)
Micro insulin	20 (45)	11 (34)
Anti–IA-2, harmonized	27 (61)	24 (75)
ICA	29 (66)	28 (88)
Anti-ZnT8	32 (73)	24 (75)
Median glycated hemoglobin level (IQR) — %	5.2 (4.9–5.4)	5.3 (5.1–5.4)

* Percentages may not total 100 because of rounding. GAD65 denotes glutamic acid decarboxylase 65, IA-2 islet antigen 2, ICA islet-cell autoantibody, IQR interquartile range, and ZnT8 zinc transporter 8.
† Participants in this category may have had more than one sibling with type 1 diabetes.
‡ Shown are the autoantibodies for which participants were positive at the time of randomization. All participants were positive for at least two autoantibodies before randomization.

Lan–DeMets stopping rules were used.[20] Data were analyzed according to the intention-to-treat principle. Tests of significance reported herein are two-sided, with a threshold of significance of 0.05. The interim assessment had a negligible effect on the threshold of significance for the final analysis (one-sided P=0.0247), and therefore fixed-sample significance levels are reported. All confidence intervals reported are 95% confidence intervals. Subgroup analyses were prespecified but were not adjusted for multiple testing. Flow-cytometry data were analyzed by means of analysis of variance at four time points. Statistical analyses were performed with either TIBCO Spotfire S+ Workbench, version 8.2 (TIBCO), or SAS software, version 9.4 (SAS Institute).

RESULTS

PARTICIPANTS

Of the 112 potential participants who were screened for eligibility, 76 underwent randomization — 44 to the teplizumab group and 32 to the placebo group (Fig. S2 in the Supplementary Appendix). The randomization process resulted in unequal numbers of participants in the treatment groups, perhaps because of the small number of enrolled participants (<4) at some study sites, randomization stratification, or other, unclear factors. Before enrollment, all participants were positive for at least two autoantibodies, and 71% were positive for three or more autoantibodies. The treatment groups were generally well balanced with regard to baseline characteristics (Table 1, and Table S2 in the Supplementary Appendix). The majority of participants (55 [72%]) were children (<18 years), most were white, and more than half were siblings of patients with type 1 diabetes. Of the 55 participants who were younger than 18 years of age, 47 had a confirmed dysglycemic oral glucose-tolerance test result before randomization. Of the participants who underwent randomization after a single dysglycemic test result, 2 had diabetic and 6 had normal oral glucose-tolerance test results on the day of randomization. These participants were included in the intention-to-treat analysis, which was adjusted for the results of the blinded oral glucose-tolerance test before randomization.

In total, 93% of participants in the teplizumab group (41 of 44) and 88% of participants in the placebo group (28 of 32) completed the 14-day course of the assigned trial agent. The median total dose of teplizumab administered was 9.14 mg per square meter (interquartile range, 9.01 to 9.37). Three participants in the teplizumab group and 4 participants in the placebo group did not complete the trial regimen; the reasons were laboratory abnormalities (4 participants), an inability to have intravenous access established (2), or rash (1). The median follow-up duration was 745 days (range, 74 to 2683). The duration of follow-up was more than 3 years in 57 participants (75%). Type 1 diabetes was diagnosed in 42 participants (55%).

EFFICACY

Treatment with a single course of teplizumab delayed the time to diagnosis of type 1 diabetes

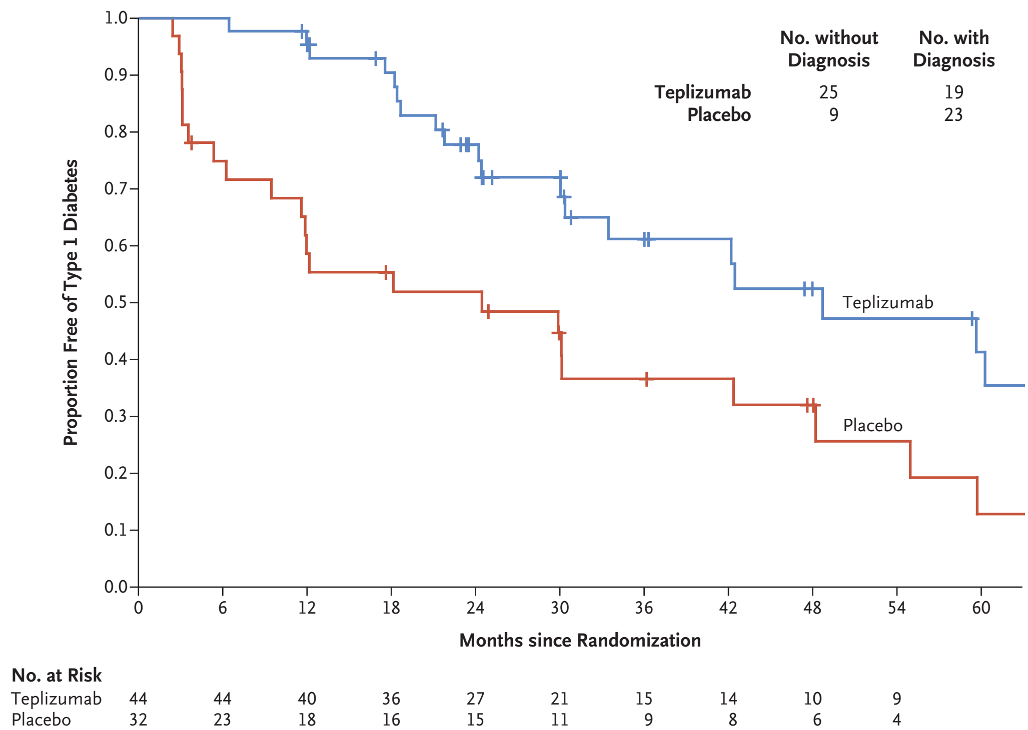

Figure 1. Effects of Teplizumab on Development of Type 1 Diabetes.

Shown are Kaplan–Meier estimates of the proportions of participants in whom clinical diabetes was not diagnosed. The overall hazard ratio was 0.41 (95% confidence interval [CI], 0.22 to 0.78; two-sided P=0.006 by adjusted Cox proportional-hazards model). The median time to diagnosis of type 1 diabetes was 48.4 months in the teplizumab group and 24.4 months in the placebo group. The numbers of participants with or without a diagnosis of clinical type 1 diabetes (upper right) represent data at the conclusion of the trial. Tick marks indicate censored data.

(Fig. 1): 19 (43%) of the 44 participants who received teplizumab and 23 (72%) of the 32 participants who received placebo had type 1 diabetes diagnosed. The annualized rates of diagnosis of type 1 diabetes were 14.9% per year in the teplizumab group and 35.9% per year in the placebo group. The median time to diagnosis was 48.4 months in the teplizumab group and 24.4 months in the placebo group (hazard ratio, 0.41; 95% confidence interval [CI], 0.22 to 0.78; two-sided P=0.006). The hazard ratio remained significant when adjusted for prespecified covariates of age, the results of the second oral glucose-tolerance test before randomization, or the presence of anti-GAD65 antibody.

The percentage of participants with progression to clinical type 1 diabetes in the overall trial population was greater in the first year after trial entry (17 of the 42 participants with progression, 40%) than in year 2 (10 participants, 24%), year 3 (6 participants, 14%), or year 4 (5 participants, 12%) (Table S3 in the Supplementary Appendix). The largest effect of teplizumab treatment was found in the first year: diabetes was diagnosed in only 3 of 44 participants (7%) in the teplizumab group, in contrast to 14 of 32 participants (44%) in the placebo group (unadjusted hazard ratio, 0.13; 95% CI, 0.05 to 0.34).

SAFETY

Teplizumab treatment was associated with adverse events, which are listed in Table 2. Similar to findings in previous trials of teplizumab in patients with new-onset type 1 diabetes, the lymphocyte count decreased to a nadir on day 5 (total decrease, 72.3%; interquartile range, 82.1 to 68.4; P<0.001) (Fig. 2A).[7,8] A total of 15 (75%) of the 20 grade 3 events in the teplizumab group involved lymphopenia during the first 30 days after administration. Lymphopenia resolved by day 45

Table 2. Adverse Events during Active Follow-up.*

Adverse Event Category	Teplizumab		Placebo	
	Events (N=112)	Participants (N=44)	Events (N=23)	Participants (N=32)
	no.	*no. (%)*	*no.*	*no. (%)*
Blood or bone marrow†	45	33 (75)	2	2 (6)
Dermatologic or skin†	17	16 (36)	1	1 (3)
Pain	11	5 (11)	5	3 (9)
Infection	8	5 (11)	5	3 (9)
Gastrointestinal	5	4 (9)	3	3 (9)
Metabolic or laboratory	7	4 (9)	2	2 (6)
Pulmonary or upper respiratory	6	4 (9)	0	0
Constitutional symptoms	3	2 (5)	0	0
Allergy or immunologic	2	2 (5)	0	0
Cardiac, general	1	1 (2)	1	1 (3)
Endocrine	0	0	2	2 (6)
Vascular	1	1 (2)	1	1 (3)
Neurologic	1	1 (2)	0	0
Ocular or visual	1	1 (2)	0	0
Musculoskeletal or soft tissue	2	1 (2)	0	0
Hepatobiliary or pancreatic	0	0	1	1 (3)
Syndrome	1	1 (2)	0	0
Hemorrhage or bleeding	1	1 (2)	0	0

* Events listed were attributed as possibly, probably, or definitely related to the trial agent by the trial-site investigator.
† The frequency of this type of event differed significantly between the two groups (P<0.001).

in all participants except one; in that participant, the lymphocyte counts returned to the normal range on day 105. A spontaneously resolving rash, as previously noted, occurred in 16 (36%) of participants who received teplizumab.[8] The rates of infection were similar in the two treatment groups.

Anti-CD3 monoclonal antibody treatment has been associated with Epstein–Barr virus (EBV) reactivation.[21,22] At trial entry, 30 participants (39%; 16 in the teplizumab group and 14 in the placebo group) had antibodies against EBV. At weeks 3 through 6 after receipt of the trial regimen, there was quantifiable EBV DNA in whole blood in 8 of the seropositive participants — all in the teplizumab group — one of whom had symptoms of pharyngitis, rhinorrhea, and cough on day 38. In these participants, the EBV DNA levels decreased to below the level of quantification between day 43 and day 134 (mean day 77). At trial entry, 17 participants (10 in the teplizumab group and 7 in the placebo group) had antibodies against cytomegalovirus (CMV). One participant in the teplizumab group who was CMV-seropositive had detectable levels of CMV DNA at day 20, but CMV DNA was undetectable by day 42.

CHANGES IN IMMUNE-CELL SUBSETS

An increased frequency of TIGIT+KLRG1+EOMES+ CD8+ T cells, associated with T-cell unresponsiveness, has previously been reported among patients with new-onset diabetes who had a response to teplizumab.[12,13] To determine whether treatment with teplizumab in the current prevention trial was associated with similar changes, we analyzed the frequency of KLRG1+TIGIT+CD8+ T cells among the total CD3+ T cells in the two treatment groups. These cells were more common at months 3 and 6 than at baseline in par-

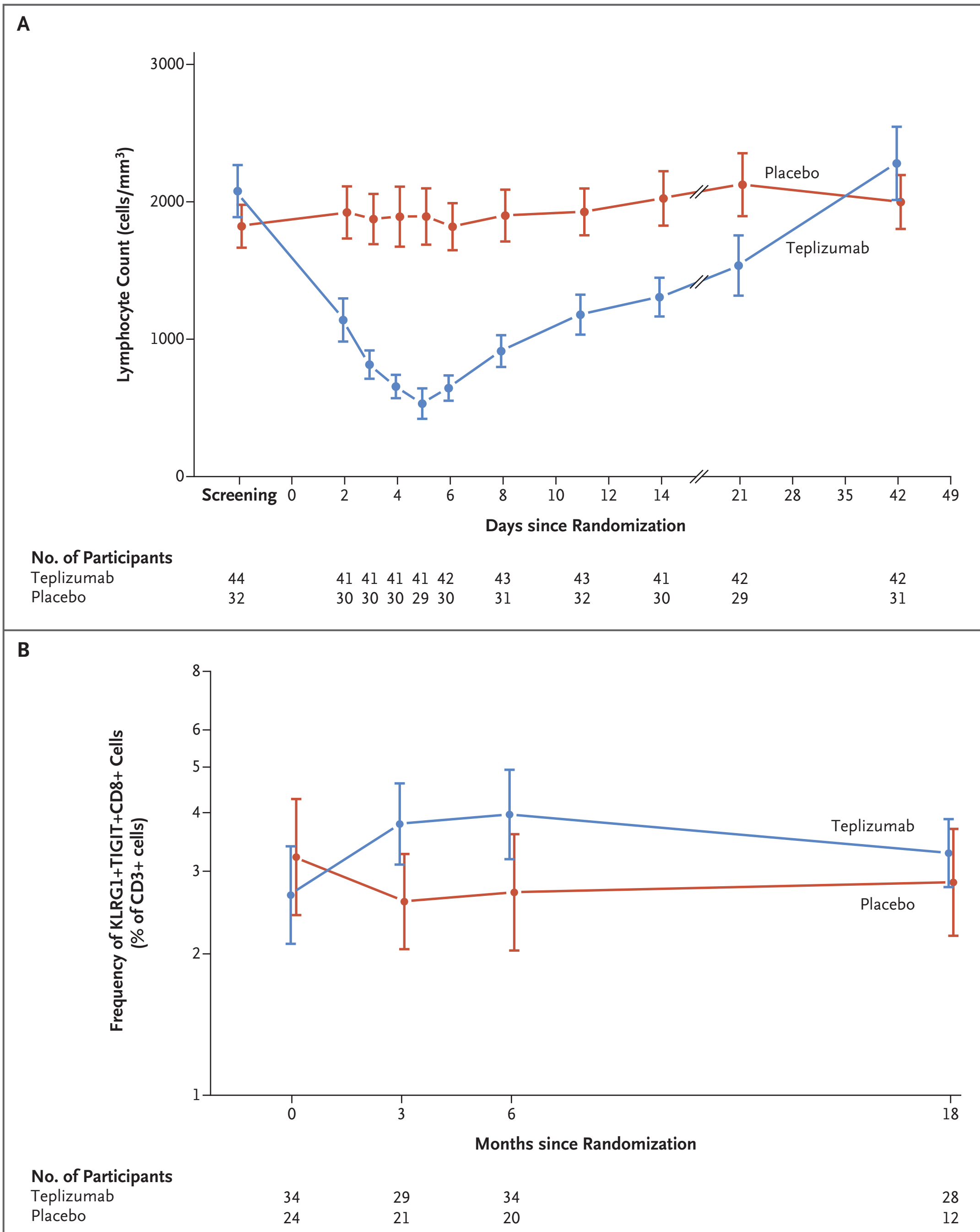

Figure 2. Changes in T-Cell Subsets in the Treatment Groups.

Panel A shows the absolute lymphocyte counts in the treatment groups over the first 7 weeks after enrollment. Panel B shows the frequency of KLRG1+TIGIT+CD8+ T cells as a percentage of total CD3+ T cells in the teplizumab and placebo groups. The estimates of the percentage differences between the teplizumab group and the placebo group are 46.5% at 3 months (95% CI, 8.23 to 98.4), 49% at 6 months (95% CI, 4.13 to 113), and 15.9% at 18 months (95% CI, −14.2 to 56.4). The analysis was performed with log-transformed values by analysis of covariance and corrected for the baseline values. In both panels, means and 95% confidence intervals are shown.

ticipants who received teplizumab (mean, 3.79% [95% CI, 3.1 to 4.62] at month 3 and 3.97% [95% CI, 3.18 to 4.94] at month 6, vs. 2.67% [95% CI, 2.1 to 3.39] at baseline), and the levels at months 3 and 6 were higher than those in participants who received placebo (mean, 2.59% [95% CI, 2.05 to 3.27] at month 3 and 2.71% [95% CI, 2.03 to 3.6] at month 6) (Fig. 2B, and Figs. S3 and S4 in the Supplementary Appendix). In contrast, the frequency of CD4+ regulatory T cells or KLRG1–TIGIT–CD8+ T cells did not differ significantly between the two treatment groups, which suggested that there was selectivity in the effect of teplizumab[23,24] (Fig. S3 in the Supplementary Appendix).

SUBGROUP ANALYSIS

In prespecified analyses, we compared the effects of teplizumab in subgroups based on age, HLA type, pretreatment C-peptide and glucose levels during the oral glucose-tolerance tests, and autoantibodies (Fig. 3). Among the 43 participants in the teplizumab group for whom data were available, 21 (49%) had HLA-DR3 and 28 (65%) had HLA-DR4 major histocompatibility complex (MHC) molecules. The presence of HLA-DR4 and absence of HLA-DR3 were associated with more robust responses to teplizumab (hazard ratio, 0.20 [95% CI, 0.09 to 0.45] and 0.18 [95% CI, 0.07 to 0.45], respectively, without adjustment for multiplicity) (Figs. S5 and S6 in the Supplementary Appendix). The response to teplizumab as compared with placebo was greater among participants without anti–zinc transporter 8 (ZnT8) antibodies than among those with these antibodies (hazard ratio, 0.07; 95% CI, 0.02 to 0.26) (Fig. S7 in the Supplementary Appendix). The presence or absence of other autoantibodies was not associated with clinical response. The response to teplizumab was also greater among participants whose C-peptide responses to the oral glucose-tolerance test at baseline were below the median (1.75 nmol per liter) than among those whose responses were above the median (hazard ratio, 0.19; 95% CI, 0.08 to 0.47) (Fig. S8 in the Supplementary Appendix).

DISCUSSION

In this phase 2 trial, a single course of teplizumab significantly slowed progression to clinical type 1 diabetes in high-risk, nondiabetic relatives of patients with diabetes and had at least two autoantibodies and abnormal results of an oral glucose-tolerance test at trial entry. The median delay in the diagnosis of diabetes was 2 years; at the conclusion of the trial, the percentage of diabetes-free persons in the teplizumab group (57%) was double that in the placebo group (28%). The safety analysis revealed expected adverse events of rash and transient lymphopenia among both children and adults. The delay of progression to diabetes is of clinical importance, particularly for children, in whom the diagnosis is associated with adverse outcomes, and given the challenges of daily management of the condition.[2,4] Our findings support the notion that type 1 diabetes is a chronic T-cell–mediated disease and suggest that immunomodulation before the development of clinical disease can be useful.[6,25]

The effects of teplizumab were greatest in the first 3 years after administration. Among the participants in whom diabetes was diagnosed, 41% had the disease within the first year after randomization, and the risk was lowest at that time for those exposed to teplizumab. The relatively rapid rate of progression to clinical diabetes in the placebo group reflects the very high risk among children with autoantibodies.[5,26,27] Indeed, our observations among young persons who did not yet have clinical disease reflect the likely progression when two or more autoantibodies and dysglycemia are found and are consistent with our report of high rates of beta-cell death in these persons.[26-28] Preclinical studies suggested that an active autoimmune response is needed for the actions of an anti-CD3 monoclonal antibody[29,30]; thus, earlier interventions (i.e., during stage 1) may be less efficacious. Consistent with such observations, the response to teplizumab was greatest among participants with C-peptide responses that were below the median. We speculate that the efficacy during the period before diagnosis supports the development of an active screening program to identify persons who are at extremely high risk for disease progression.

Our data suggest that responses to teplizumab differ on the basis of characteristics of the participants. The absence of one type 1 diabetes–associated MHC allele, HLA-DR3, but the presence of HLA-DR4, as well as the absence of anti-ZnT8 antibodies identified the persons most likely to have a response. The MHC may modulate responsiveness to teplizumab through its effect

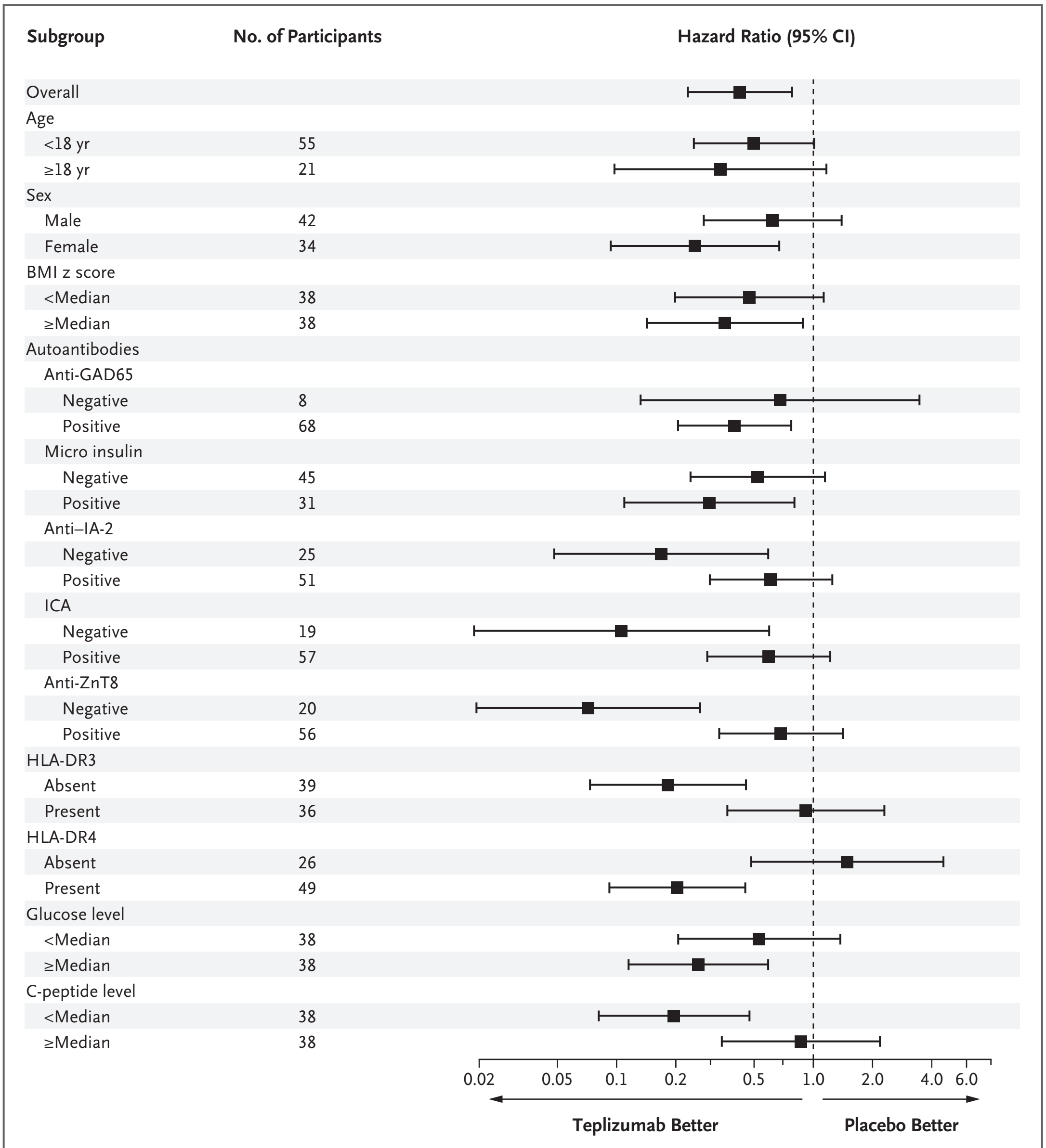

Figure 3. Subgroup Analysis of Responses to Teplizumab.

The forest plot shows the hazard ratios and 95% confidence intervals for a diagnosis of type 1 diabetes in the teplizumab group as compared with the placebo group for the two categories of each baseline feature. The Cox model was adjusted for age, with the exception of the interaction test for age (<18 years vs. ≥18 years), but was not adjusted for multiple testing. BMI denotes body-mass index, GAD65 glutamic acid decarboxylase 65, IA-2 islet antigen 2, ICA islet-cell autoantibody, and ZnT8 zinc transporter 8.

on the T-cell repertoire, perhaps altering T-cell activation status and susceptibility to the effects of the drug. We speculate that anti-ZnT8 antibodies may identify persons with a more fulminant immune response or other features that make their T cells less susceptible to teplizumab.

The transient decline in lymphocyte counts with teplizumab treatment most likely reflects egress from the peripheral blood.[31,32] Our flow-cytometry studies may suggest that teplizumab treatment causes changes in the phenotype of CD8+ T cells; we have previously associated these changes with a nonresponsive or "exhausted" phenotype.[13] These CD8+ T cells are not, however, inactive, since the few participants with detectable EBV and CMV DNA had rapid clearance of these DNA loads.[33-35] The resolution of EBV and CMV activation and the absence of an increased rate of infectious adverse events lead us to hypothesize that the duration of the functional effects of teplizumab on T cells may be affected by their avidity for autoantigens, viral antigens, or other antigens. The effects may be short-lived in T cells that have high avidity for viral antigens such as those associated with EBV but longer-lived in autoreactive T cells, which have lower avidity. Future studies with antigen-reactive T cells will be needed to address this hypothesis.

Our trial had certain limitations. The cohort was relatively small, and the estimated power was limited. The participants were relatives of patients with type 1 diabetes, and we do not know whether these findings will be generally applicable to persons who do not have first-degree relatives with diabetes and who appear to be at risk for type 1 diabetes. Although it reflected the known incidence of disease, our trial population was overwhelmingly made up of non-Hispanic white participants. The drug was given for only one course, and although repeated dosing may provide additional benefits and capture more persons with active disease or prolong the therapeutic effect, this strategy was not tested in this trial.[7,11] We have not fully assessed the potential development of antibodies to teplizumab, which would be a concern. An assay to detect such autoantibodies has not yet been fully vetted and validated. In previous trials, antidrug antibodies have been found in approximately 20% to 55% of teplizumab-treated participants after the first course, but the effects on the immunologic or clinical outcomes are not clear.[10,36]

In conclusion, in our trial, a 2-week course of treatment with teplizumab delayed the diagnosis of clinical type 1 diabetes in high-risk participants.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Herold KC, Bundy BN, Long SA, et al. An anti-CD3 antibody, teplizumab, in relatives at risk for type 1 diabetes. N Engl J Med. DOI: 10.1056/NEJMoa1902226

Supplementary Appendix

Table of Contents

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Walter & Eliza Hall Institute of Medical Research, Parkville, Australia: Peter G. Colman, John Wentworth, Marika Bjorasen, Candice Breen, Spiros Fourlanos, Felicity Healy, Leanne Redl.

Yale University, New Haven, Connecticut: Kevan Herold, Laurie Feldman, Jennifer Sherr, Robert Sherwin, William Tamborlane, Stuart Weinzimer.

Supplementary methods:

Patient identification: Potential participants were identified in the TrialNet Pathway to Prevention (PTP) study. The PTP study enrolled first degree relatives of patients with T1D, ages 1-45, and up to age 20 in second- or third- degree relatives, evaluated diabetes autoantibodies to microinsulin (mIAA), glutamic acid decarboxylase-65 (GAD), and insulinoma-associated antigen-2 (IA-2, or ICA512). Islet cell (ICA) and zinc transporter 8 (ZnT8) autoantibodies were measured if at least 1 other antibody tested positive.

Drug Dosing: Those assigned to active study drug received teplizumab at a total dose of 9,034 μg/m^2 over 14 days as described in the Methods. Participants randomized to the placebo arm received a 14-day course of matching IV saline. Participants received ibuprofen and diphenhydramine prior to infusions on the first 5 days, and further dosing with ibuprofen, diphenhydramine and/or acetaminophen thereafter as needed for symptomatic relief. Protocol defined stopping criteria for study drug infusions were followed. During the entire study, all subjects had interim contact with study personnel for formal inquiry about adverse events and symptoms of diabetes.

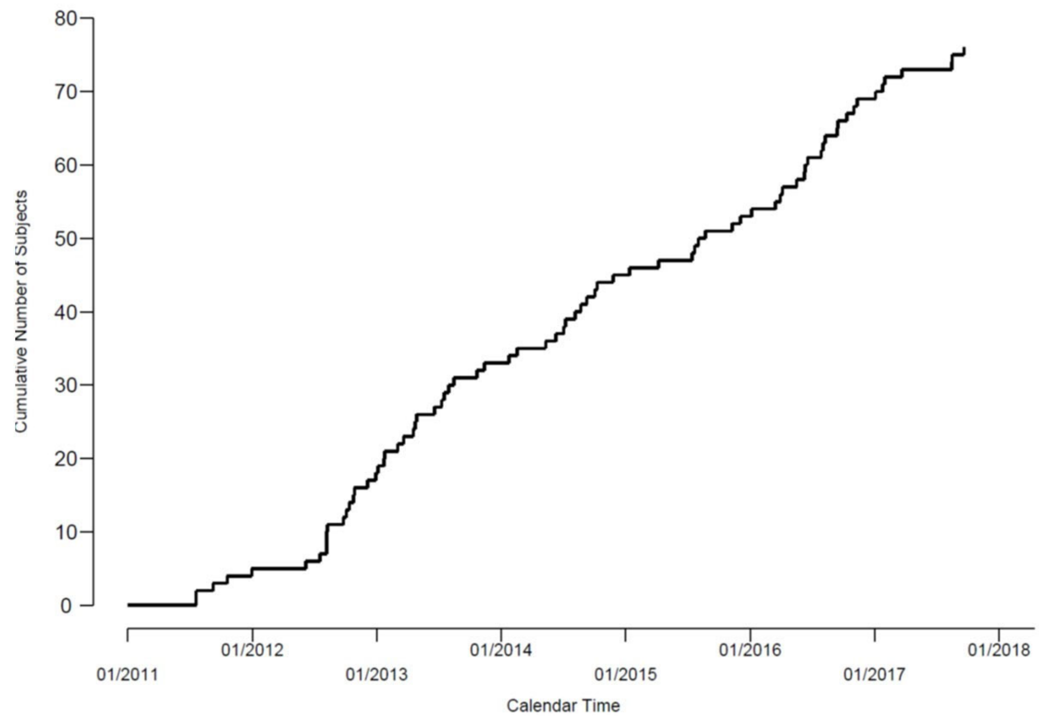
Laboratory Methods: mIAA, GAD-65Ab, ICA-512Ab, ZnT8A were measured using radio-immunobinding assays at the Barbara Davis Diabetes Center, Anschultz CO, and ICA using indirect immunofluorescence at the University of Florida at Gainesville. C-peptide, glucose and HbA1c were measured at the Northwest Research Laboratory, Seattle, WA. C-peptide was measured from frozen plasma by two-site immunoenzymometric assay (Tosoh Bioscience, South San Francisco, CA) at the HbA_{1c} was measured using ion-exchange high performance liquid chromatography (Variant II, Bio-Rad Diagnostics, Hercules, CA). Reliability coefficients for each assay were above 0.99 from split duplicate samples. EBV and CMV viral loads were measured in whole blood at the University of Colorado using previously described methods.[1] Positive viral loads were designated as > 500 copies of viral DNA.[2,3]

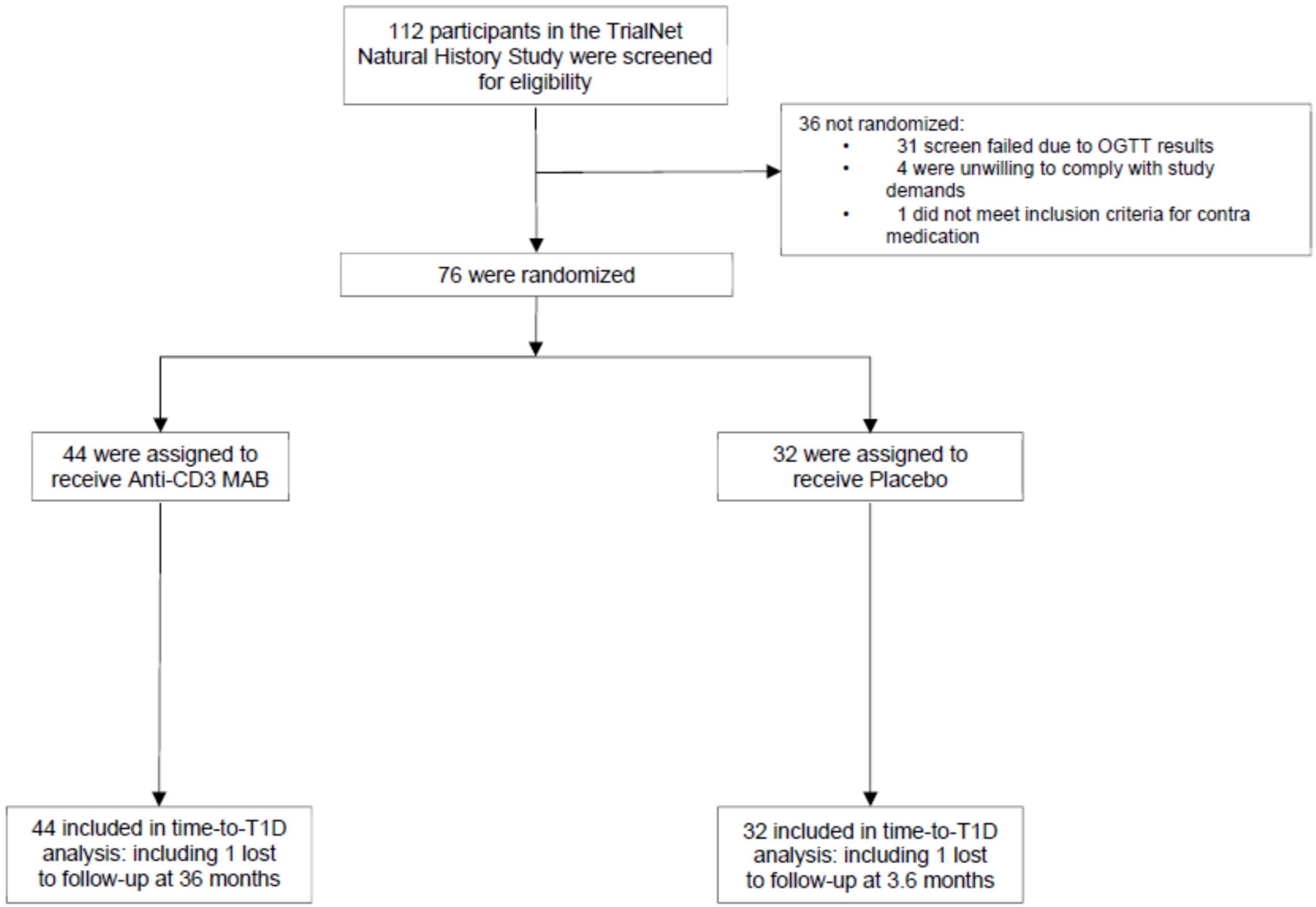
Flow cytometry: Peripheral blood mononuclear cells (PBMC) were processed and stored at the NIDDK repository. Frozen vials of PBMC were sent to Benaroya Research Institute for analysis by flow cytometry with antibody panels shown in Supplementary Table 1. T-cell phenotyping was performed on PBMC as previously described on an LSR-Fortessa (BD Biosciences) with FACS Diva software and analyzed with FlowJo software version 9.5 (Tree Star, Ashland, OR). The frequency of CD8+ T-cells that were TIGIT+KLRG+CD57-, TIGIT-KLRG1-CD57- , or CD4+CD127^{lo}Foxp3+ (CD4+Tregs) were determined as described previously.[4] The quadrants were placed based on staining controls.

Trial oversight: The results were shared with Provention Bio prior to publication. The statistical analysis plan is available at NEJM.org. The TrialNet Coordinating Center gathered, analyzed, and vouches for the data. The authors are fully responsible for the content and editorial decisions regarding the manuscript. The authors approved the final version for submission.

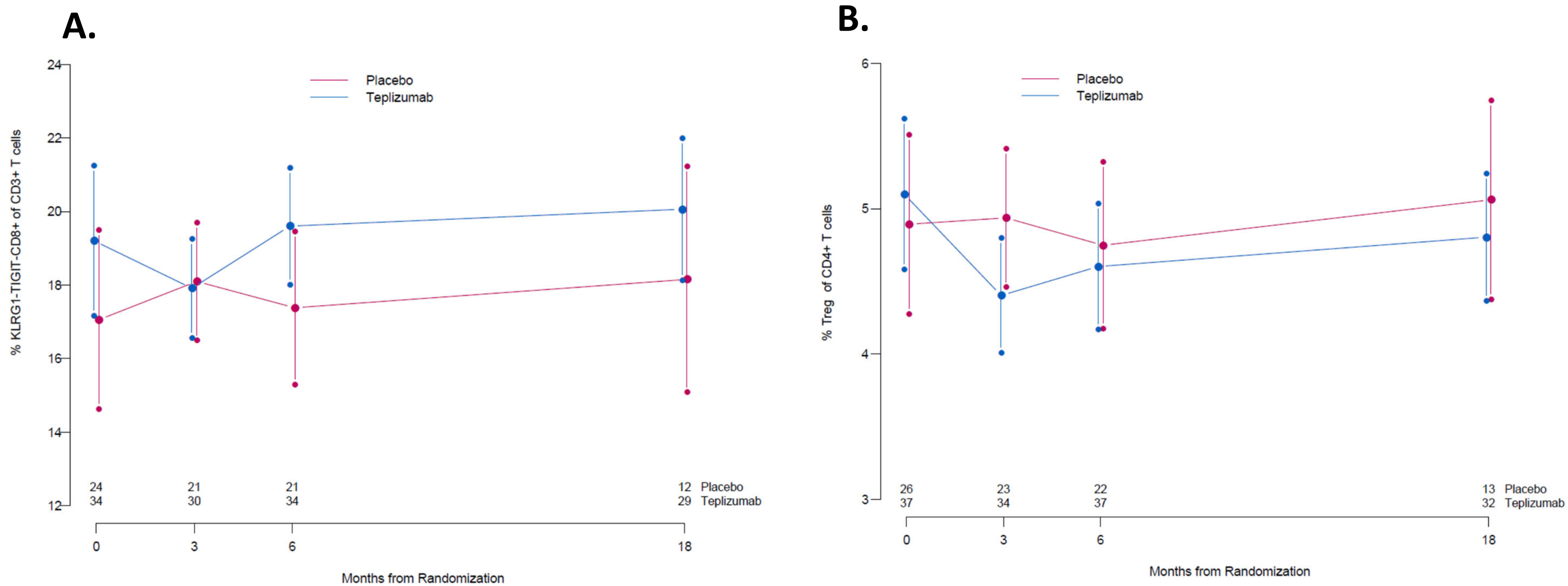
Participating sites: For screening, infusion, and follow up: Barbara Davis Center for Childhood Diabetes, Benaroya Research Institute, Indiana University - Riley Hospital for Children, Klinikum rechts der Isar, Technical University Munich, Germany, , The Children's Mercy Hospital, The Hospital for Sick Children, University of California - San Francisco, University of Florida, University of Iowa, Stead Family Children's Hospital, University of Miami, University of Minnesota, USF Diabetes Center, Vanderbilt Eskind Diabetes Clinic, Yale University School of Medicine; Follow up: Joslin Diabetes Center, University of Utah, University of Pittsburgh, GHS - Pediatric Endocrinology, Trustees of Dartmouth College, Endocrinology Specialist/Greenville Health System, Washington University, Columbia University, University of Texas Southwestern, UNC Chapel Hill, Stanford University, Children’s Hospital of Los Angeles For screening: University of Chicago, University of Cambridge, Walter and Eliza Hall Institute. The TrialNet network of sites was used for screening into the Pathway to Prevention protocol.

Supplementary Appendix Figure S1: Enrollment in the Trial

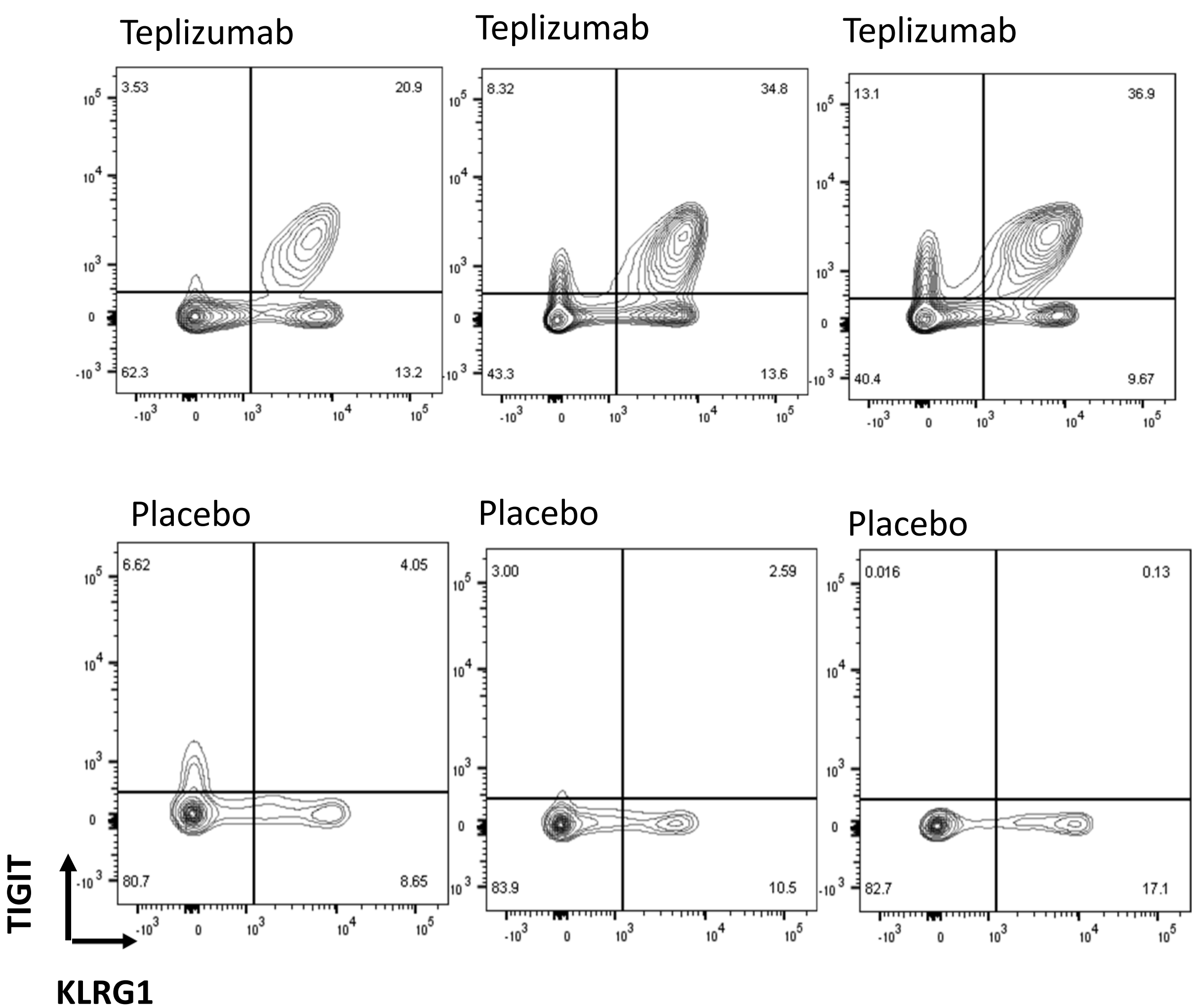




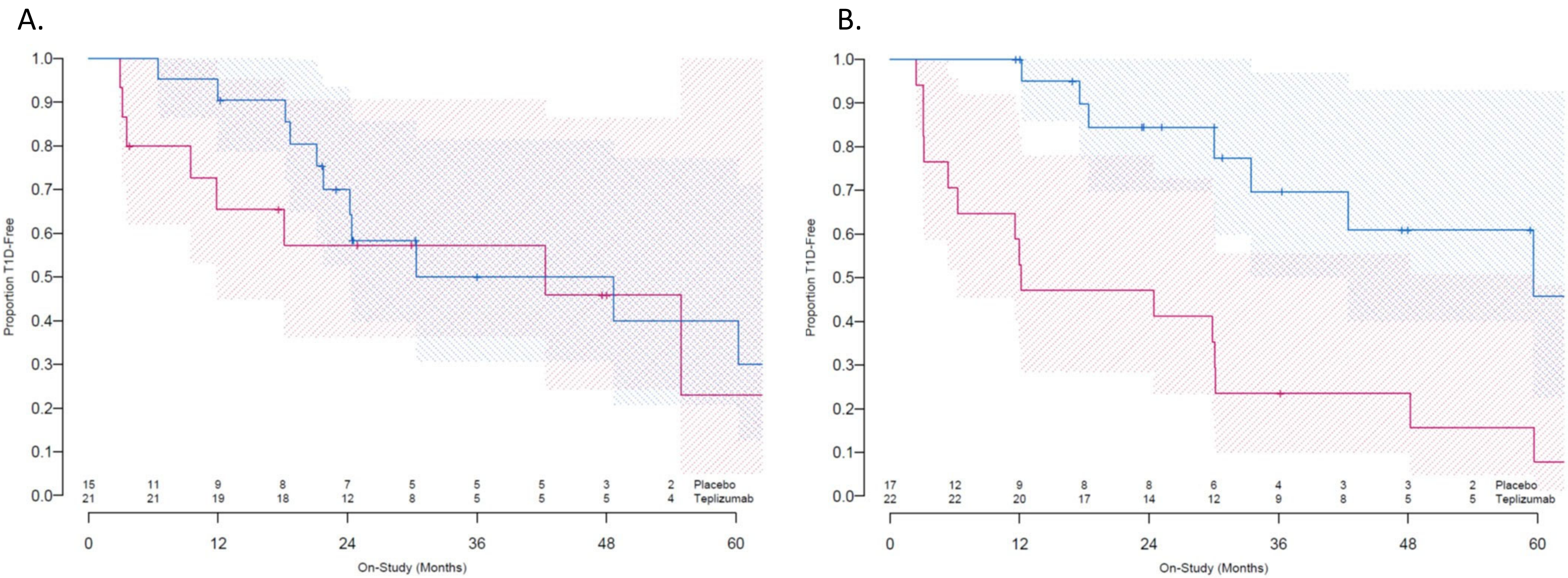
Supplementary Appendix Figure S2: Screening, enrollment and follow-up of the participants: A total of 112 participants from the TrialNet Natural History Study were screened for eligibility (see Appendix for a listing of study sites). Seventy-six of the participants were randomized to the drug or placebo arms. They were infused with study drug at one of 14 TrialNet sites and followed, as per study protocol at one of 33 sites. All randomized participants are included in the analysis.



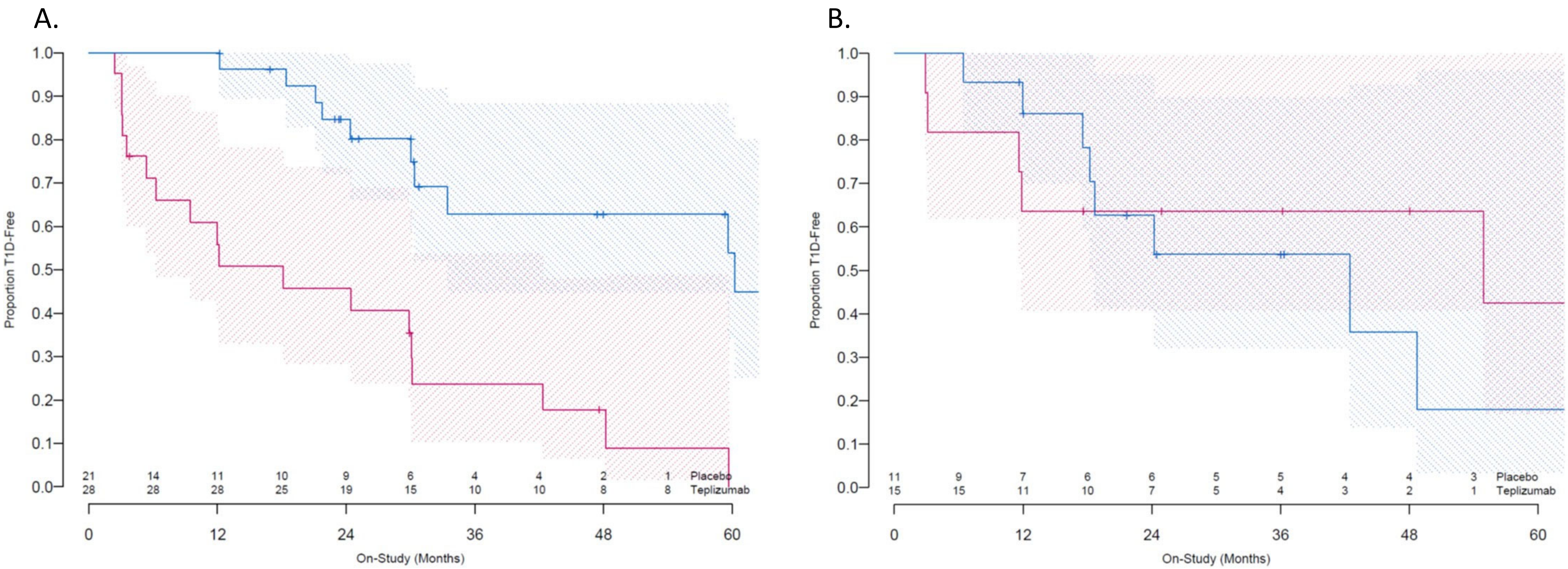
Supplementary Appendix Figure S3: The frequency of (A) KLRG1-TIGIT-CD8+ T cells (of total CD3+T cells) and (B) CD4+ Tregs (CD4+CD25+CD127^{lo}) in the teplizumab and placebo treated groups. The mean±95% CI are shown. The analysis was performed by ANCOVA and corrected for the baseline values. The numbers along the X axis indicate the number of samples analyzed.



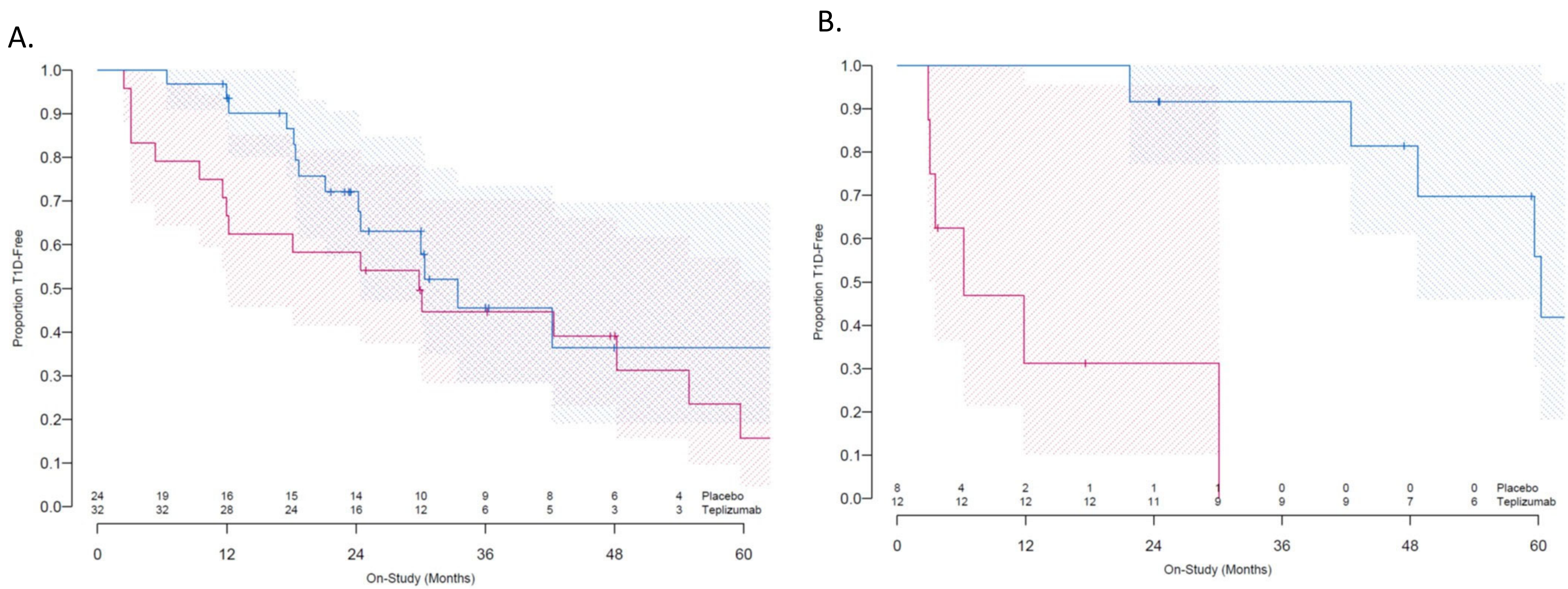
Supplementary Appendix Figure S4: FACS contour plots showing staining of TIGIT (Y axis) vs KLRG1 (X axis).
Electronic gates were placed on live CD8+CD57- T cells and the expression of KLRG1 and TIGIT are shown in peripheral blood cells from 3 subjects treated with teplizumab (top row) and 3 participants treated with placebo on samples that were acquired at month 3. The numbers refer to the proportion of the total gated cells in each quadrant. The quadrants were placed based on staining controls.



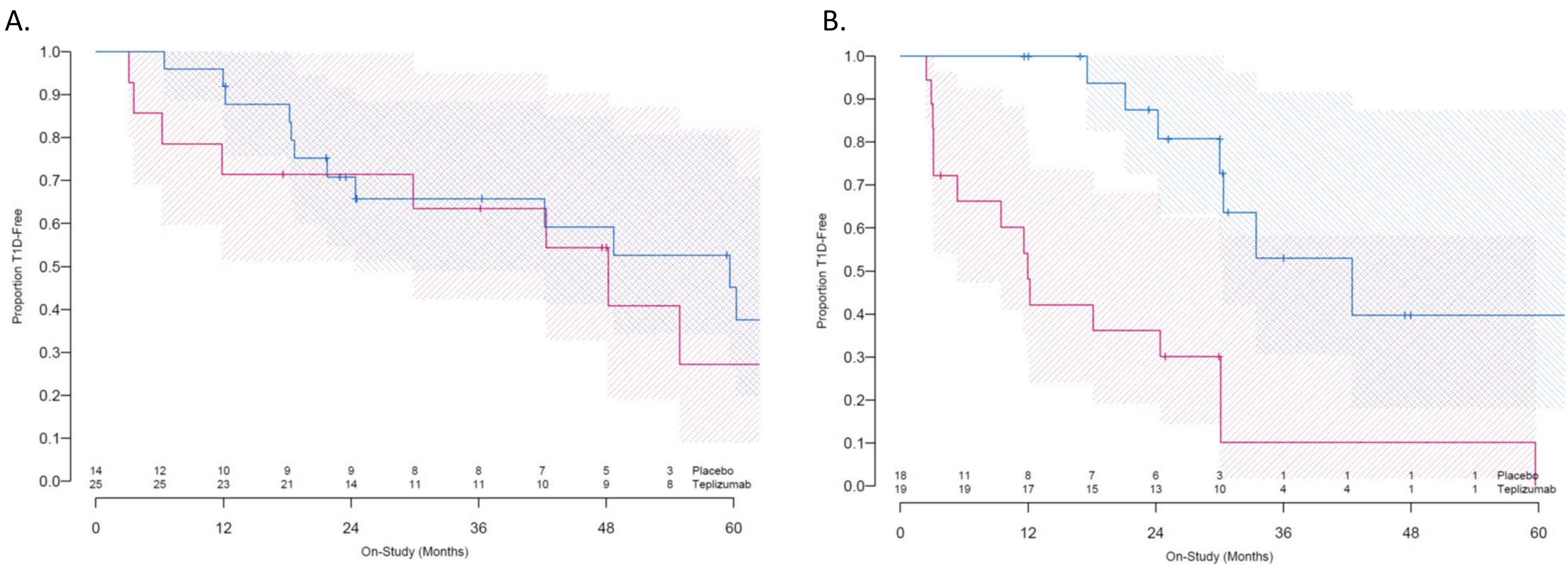
<u>Supplementary Appendix Figure S5: The effects of teplizumab treatment in participants with (A) and without (B) HLA-DR3.</u> The absence of HLA-DR3 (Hazard ratio:0.181 for negative, 0.907 for positive) was associated with response to teplizumab. (For each graph ----- = teplizumab, ----- = placebo, + = censored. The number of participants at risk are shown along the X axis. The shaded areas represent the 95% CIs.)



Supplementary Appendix Figure S6: The effects of teplizumab treatment in participants with (A) and without (B) HLA-DR4. The presence of HLA-DR4 (Hazard ratio: 1.47 for negative, 0.201 for positive) was associated with response to teplizumab. (For each graph ----- = teplizumab, ----- = placebo, + = censored. The number of participants at risk are shown along the X axis. The shaded areas represent the 95% CIs.)



<u>Supplementary Appendix Figure S7: The effects of teplizumab treatment in participants with (A) and without (B) anti-ZnT8 antibodies at randomization</u>. The absence of anti-ZnT8 antibody (Hazard ratio: 0.064 for negative, 0.831 for positive) was associated with response to teplizumab. (For each graph ----- = teplizumab, ----- = placebo, + = censored. The number of participants at risk are shown along the X axis. The shaded areas represent the 95% CIs.)



Supplemental Appendix, Figure S8: The effects of teplizumab treatment in participants whose C-peptide area under the curve during the oral glucose tolerance test at randomization was above (A) or below (B) the median (1.75 nmol/L). C-peptide responses below the median were associated with responses to teplizumab. (Hazard ratio=0.855 above (A) and 0.194 below (B) the median). (For each graph ----- = teplizumab, ----- = placebo, + = censored. The number of participants at risk are shown along the X axis. The shaded areas represent the 95% Cis.)

Supplementary Appendix Table S1: Monoclonal antibodies used for flow cytometry

Marker	Format	Clone	Vendor
CD56	BUV395	NCAM16.2	Becton Dickinson
CD45RA	BUV737	HI100	Becton Dickinson
Ki67	BV421	Ki-67	BioLegend
CCR7	BV510	G043H7	BioLegend
CD3	BV605	OKT3	BioLegend
PD1	BV650	EH12.2H7	BioLegend
CD127	BV711	A019D5	BioLegend
CD45R0	BV786	UCHL1	Becton Dickinson
CD4	BB515	RPA-T4	Becton Dickinson
Eomes	PE	WD1928	eBiosciences
FoxP3	PE-CF594	259D/C7	Becton Dickinson
KLRG1	PE-Vio770	REA261	Miltenyi
TIGIT	APC	MBSA43	eBiosiences
CD8	Ax700	SK1	BioLegend
CD57	APC-Vio770	REA769	Miltenyi
Live/dead	BUV496	NA	Becton Dickinson

Supplementary Appendix Table S2: Additional demographic data of participants

	Teplizumab, N=44	**Placebo N=32**
Male sex No. of subjects (%)	25 (56.8)	17 (53.1)
Body Mass Index (kg/m^2)-median* Z-score BMI-median*	19.6 (17.3 – 25.4) 0.259 (-0.754 - 1.19)	21.5 (18.2 – 24.7) 0.681 (0.339 – 1.11)
Race - No. of subjects (%)		
White	44 (100.0)	30 (93.8)
African American	0 (0.0)	0 (0.0)
Asian	0 (0.0)	2 (6.2)
Ethnicity - No. of subjects (%) Non-Hispanic	43 (97.7)	31 (96.9)
Autoantibodies titer – median*		
Anti-GAD65 (harmonized)	240 (76.8 – 464)	221 (42.3 – 520)
Micro Insulin	0.0070 (0.0020 – 0.028)	0.0040 (0.0020 – 0.0168)
Anti-IA-2 (harmonized)	52 (0 – 310)	187 (26 – 253)
ICA	20 (0 – 200)	80 (20 – 160)
Zinc Transporter	0.157 (0.0133 – 0.496)	0.096 (0.028 – 0.386)
No. of Autoantibodies Positive (% of total)^		
1	1 (2.4)	0 (0.0)
2	11 (25.0)	7 (21.9)
3	12 (27.3)	5 (15.6)
4	11 (25.0)	14 (43.8)
5	9 (20.5)	6 (18.8)
C-peptide AUC Mean, OGTT (nmol/L) Median*	1.76 (1.47 – 2.18)	1.73 (1.44 – 2.36)
HLA alleles present - no. of subjects (%)†		
Neither DR3 or DR4	5 (11.6)	3 (9.4)
DR3 only	10 (23.3)	8 (25.0)
DR4 only	17 (39.5)	14 (43.8)

* Parenthetical value(s): The interquartile range is displayed with the median, and percent of subjects is displayed with the number of subjects.
^ at the time of randomization. All subjects had at least 2+ autoantibodies prior to randomization.
† Missing: HLA allele status missing for 1 teplizumab-treated subject

Year	No. of T1D*		Chi-square Test†	Hazard Ratio (95%CI)†	
	Teplizumab (%)	Placebo (%)		Cumulative	Interval
1	3 (6.8%)	14 (43.8%)	15.9	0.129 (0.0482, 0.343)	0.129 (0.0482, 0.343)
2	8 (18.2%)	2 (6.3%)	7.55	0.372 (0.169, 0.82)	1.8 (0.473, 6.88)
3	3 (6.8%)	3 (9.4%)	7.77	0.404 (0.198, 0.825)	0.58 (0.11, 3.05)
4	3 (6.8%)	2 (6.3%)	7.05	0.447 (0.23, 0.868)	0.864 (0.14, 5.33)
5	2 (4.5%)	2 (6.3%)	8.24	0.439 (0.233, 0.828)	0.359 (0.039, 3.32)

Supplementary Appendix, Table S3: Hazard ratios by yearly interval and cumulative. Frequency of clinical type I diabetes by treatment group and cumulative and interval hazard ratios (95% confidence Intervals) by year on-study. (* The number of participants developing T1D in each treatment arm during the year interval are shown. In addition, the cumulative HRs and the HR for each year interval were calculated. † Mantel- Haenszel method applied to time-to-event data, both chi- square test and hazard ratio estimate.[5] These hazard ratios are unadjusted for the enrollment/age strata. € Likelihood ratio test and hazard ratio estimate and 95% CI from the Cox model)

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1. Kroll J, Li S, Levi M, Weinberg A. Lytic and latent EBV gene expression in transplant recipients with and without post-transplant lymphoproliferative disorder. J Clin Virol 2011;52:231-5.
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5. Mantel, N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 1966 50:163-70.

Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

Protocol for: Herold KC, Bundy BN, Long SA, et al. An anti-CD3 antibody, teplizumab, in relatives at risk for type 1 diabetes. N Engl J Med. DOI: 10.1056/NEJMoa1902226

This supplement contains the following items:

1. Original Protocol (June 22, 2010), Final Protocol (June 25, 2014), & Summary of Protocol Amendments.

2. Original Statistical Analysis Plan (October 26, 2017), Final Statistical Analysis Plan (March 27, 2018), & Summary of Changes.

ANTI-CD3 MAB (TEPLIZUMAB) FOR PREVENTION OF DIABETES IN RELATIVES AT-RISK FOR TYPE 1 DIABETES MELLITUS

(Protocol TN-10)

VERSION

June 22, 2010

IND # 102, 629

Supported by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the National Institute of Allergy and Infectious Diseases (NIAID), the National Institute of Child Health and Human Development (NICHD), the National Center for Research Resources (NCRR), the Juvenile Diabetes Research Foundation International (JDRF), and the American Diabetes Association (ADA).

PREFACE

The TrialNet Type 1 Diabetes Protocol TN-10, Anti-CD3 (teplizumab) for Prevention of Diabetes in Relatives at risk for Type 1 Diabetes Mellitus, describes the background, design, and organization of the study. The protocol will be maintained by the TrialNet Coordinating Center over the course of the study through new releases of the entire protocol, or issuance of updates either in the form of revisions of complete chapters or pages thereof, or in the form of supplemental protocol memoranda.

TABLE OF CONTENTS

1. INTRODUCTION

1.1. Study Overview

Title	Anti-CD3 mAb (teplizumab) for prevention of diabetes in relatives at-risk for Type 1 diabetes mellitus
IND Sponsor	MacroGenics, Inc. Under IND 102,629
Study Supported by	National Institute of Diabetes, Digestive and Kidney Diseases
Conducted By	Type 1 Diabetes Trial Network (TrialNet)
Protocol Chair	Dr. Kevan Herold, Yale University
Accrual Objective	The study plans to enroll approximately 140 - 170 subjects over 2-3 years. The study is projected to last between 4 - 6 years, depending upon rate of enrollment and number of subjects who develop diabetes.
Study Design	The study is a 2-arm, multicenter, randomized, placebo controlled masked clinical trial. All subjects will receive close monitoring for development of type 1 diabetes.
Treatment Description	Subjects will receive teplizumab + close monitoring for development of type 1 diabetes or placebo + close monitoring for development of type 1 diabetes.
Objective	To assess the safety, efficacy, and mode of action of teplizumab for prevention of type 1 diabetes
Primary Outcome	The primary objective is to determine whether intervention with teplizumab will prevent or delay the development of T1DM in high risk autoantibody positive non-diabetic relatives of patients with T1DM.
Secondary Outcome	Secondary outcomes are to include analyses of C-peptide and other measures from the OGTT; safety and tolerability; and mechanistic outcomes.
Major Inclusion Criteria	Autoantibody positive relatives of T1DM proband with abnormal glucose tolerance. Age 8-45 years.

1.2. Statement of Purpose

This protocol describes the background, design, and organization of study of the anti-CD3 monoclonal antibody, teplizumab [hOKT3γ1(Ala-Ala)] for prevention of diabetes in relatives at very high risk for type 1 diabetes. The protocol was written by Dr. Kevan Herold, Chair of the TrialNet Anti-CD3 Protocol Committee, the TrialNet Chairman's Office at the University of Miami and the Benaroya Research Institute, and the TrialNet Coordinating Center. Significant changes that occur to this protocol during the course of the trial require the formal approval of the TrialNet Steering Committee. The study protocol, along with the required informed consent forms, will be approved by each participating institution's Institutional Review Board (IRB) or Ethics Committee/Research Ethics Board (EC/REB) at international sites.

2. BACKGROUND AND SIGNIFICANCE

2.1. Type 1 Diabetes (T1DM)

2.1.1. Definition and metabolic characteristics of Type 1 diabetes mellitus

Type 1 diabetes mellitus (T1DM) is an immune-mediated disease in which insulin-producing beta cells are completely or near completely destroyed, resulting in life-long dependence on exogenous insulin. It is a chronic and potentially disabling disease that represents a major public health and clinical concern. The number of patients being diagnosed with type 1 diabetes is increasing each year and is approaching an epidemic level in some countries that track this information (1; 2).

Compared to individuals with the more common form of diabetes, Type 2 diabetes, (where individuals retain some endogenous insulin production which is inadequate to maintain normal glucose and lipid metabolism), the metabolic impairment in T1DM is much more severe and the loss of insulin production more complete. Continuous exogenous insulin therapy is needed to prevent ketoacidosis and allow assimilation of food and to maintain life. Most likely as a consequence of the absolute deficiency of insulin, glucose counterregulation (i.e. the hormonal response to insulin induced hypoglycemia) is impaired, and therefore, hypoglycemia is a frequent complication of the disease. The occurrence of hypoglycemia limits the ability to achieve near normal glucose control. The Diabetes Control and Complications study (DCCT) showed that the long term complications could be reduced with near normal control of glucose levels but at the cost of an increased frequency of severe hypoglycemia (3). While there have been significant improvements in insulin delivery systems, such as continuous subcutaneous insulin infusions with insulin pumps, normal glucose control, particularly in children, is rarely achieved. Therefore, individuals with Type 1 diabetes remain at risk for secondary end-organ complications including visual impairment and blindness, renal failure, vascular disease and limb amputation, peripheral neuropathy, stroke, acute risk for severe hypoglycemia, and others. Moreover, at the time of diagnosis, many individuals, and children in particular, suffer significant morbidity frequently requiring ICU admission. As described below, virtually all the individuals identified for enrollment into this prevention trial will develop diabetes. Clearly, prevention of the onset of the disease itself would represent a significant advancement.

2.1.2. Natural History of Type 1 Diabetes

Much is known about the natural history of the type 1 diabetes disease process (4). Although all people are susceptible, relatives of individuals with T1DM are at much greater risk for development of the disease. In the general population, approximately 0.3 % of individuals will develop T1DM. In contrast, those with a relative with T1DM have a 5% incidence of disease – a 15 fold increase (5). Further risk stratification among family members depends upon genetic, immune and metabolic data (6).

Beta cell destruction generally begins in genetically susceptible individuals years before clinical onset(7). The autoimmune process that causes beta cell destruction is clinically silent and can only be identified by the detection of autoantibodies such as Islet Cell Antibodies (ICA), anti-glutamic acid decarboxylase (GAD)65ab, anti-ICA512ab, anti-insulin autoantibodies (mIAA) (5), and the recently described antibodies to a zinc transporter (8). Continued immune mediated beta cell destruction occurs until physiologic insulin demand cannot be met by the remaining beta cells, resulting in hyperglycemia and clinical diagnosis of T1DM (9)(10).

Based on data from the Diabetes Prevention Trial, type 1 diabetes (DPT-1), the risk for developing diabetes in relatives without the disease can be defined by the presence of autoantibodies and the degree of metabolic impairment (11-13). The DPT-1 study was one of the first large-scale prevention trials of T1DM. The aim of this trial, which tested >100,000 relatives of individuals with T1DM, was to study whether either low dose parenteral insulin or oral insulin administration would prevent the development of T1DM. The results of the DPT-1 showed that neither parenteral nor oral insulin prevented the development of T1DM, (although a secondary analysis of the data suggested some effect of oral insulin in delaying the onset of diabetes in a subgroup of subjects defined by high anti-insulin antibodies and normal glucose tolerance)(13).

Autoantibody positive subjects enrolled in the DPT-1 who had impaired or indeterminate glucose tolerance (any glucose level after ingestion of oral glucose of > 200 mg/dl and/or a glucose level 2 hours after ingestion of oral glucose of 140-200 mg/dl and/or fasting glucose between 110 – 126 mg/dl during a standard oral glucose tolerance test were at very high risk (78% over 5 years) of developing T1DM over a 5 – 6 year follow up. The risk was particularly high for individuals under the age of 18 (Figure 1).

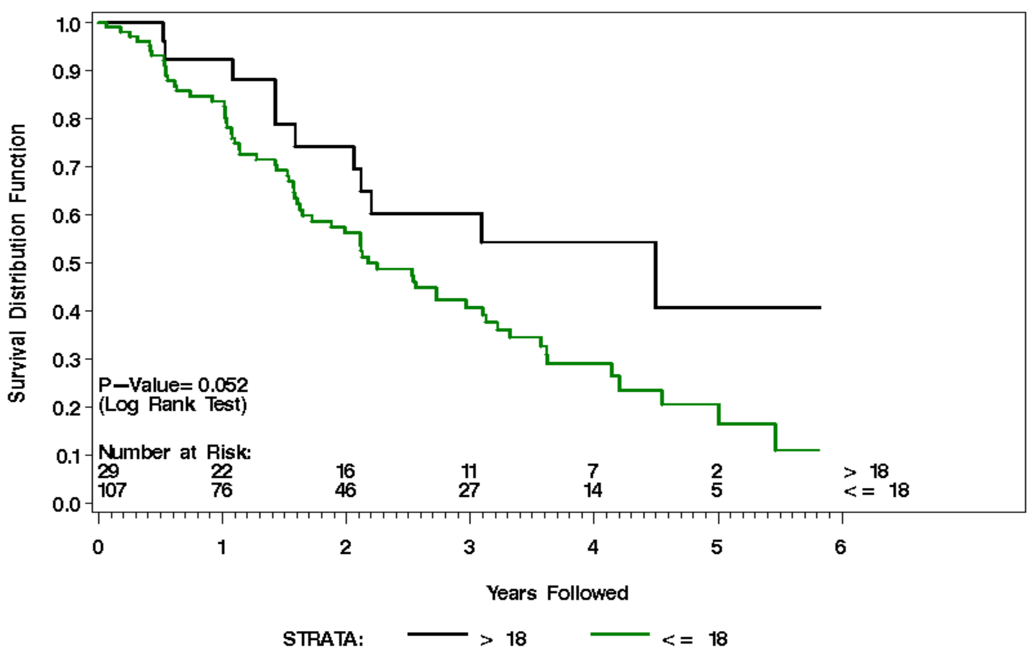


Figure 1: Risk of diabetes among individuals recruited for the DPT-1 with abnormal glucose tolerance, stratified by age≤ 18 or $\geq$ 18 yrs.

Similar results confirming the very high risk of those with abnormal glucose were found in the ENDIT (European Nicotinamide Diabetes Intervention Trial) study in which Nicotinamide failed to prevent the onset of diabetes in relatives at risk for the disease (14).

It is important to note that the diagnosis of diabetes is based on a glucose threshold that is associated with risk of secondary endorgan complications of the disease rather than the pathologic process that leads to hyperglycemia. Detailed analyses of metabolic function in individuals who do and do not progress to diabetes in the DPT-1 have been published (10; 15; 16). These studies have identified the progressive loss of stimulated C-peptide responses to a mixed meal over time in high risk individuals. The differences between the responses in the prediabetic period and after the diagnosis of diabetes are modest but statistically significant. These studies describe a progressive predictable loss of beta cell function rather than a precipitous change. In addition, they also suggest that once metabolic impairment has occurred, the risk is extremely high. These combined immunologic and metabolic studies suggest to us that those individuals differ from those in whom the diagnosis of T1DM has been made only in the time of progression.

There have been no therapies tested to date which are aimed only at those at very high risk for development of T1DM (~80% as described above). The previous DPT-1 and ENDIT studies enrolled subjects with a broad range of risk – the overall 5-year risk in the target population was between 50-60%. However, in newly diagnosed subjects, there is reason to believe that individuals with more beta cell function may show a better response to interventions. Earlier studies with Cyclosporine A suggested that response to immune therapies is greatest in those with higher levels of insulin secretion at the time of diagnosis of T1DM (17). In a recently published study of another non-FcR binding anti-CD3 mAb, Keymeulen et al found that clinical responses to drug were greatest in those in the upper half of C-peptide responses at the time of study entry (18).

Therefore, the rationale for this study is that individuals with immunologic markers of T1DM and abnormal glucose tolerance are at very high risk for progression to overt disease. They have a condition that differs from overt diabetes only in the duration of the autoimmune process that results in beta cell destruction. Intervention at the “prediabetic” stage is likely to be more effective than intervention in those in whom frank hyperglycemia has developed and beta cell function has deteriorated further because insulin production is greater before compared to after the diagnosis.

2.2. Development of Teplizumab

The Fc-engineered teplizumab [hOKT3γ1 (Ala-Ala)] was developed as an approach to mitigate the adverse effects of OKT®3 resulting from Fc/FcR engagement (19). OKT®3 produces profound, transient T-cell depletion in vivo. It also activates T cells, is strongly mitogenic, and its use in vivo is associated with severe cytokine-release syndrome (incidence >90%). The cytokine-release syndrome induced by OKT®3 is characterized by fever, chills, nausea, vomiting and other symptoms, and usually requires corticosteroid therapy to suppress. OKT®3 also is associated with a small incidence of EBV lymphomas (~1%-2%). T-cell activation is strongly facilitated by the interaction of Fc component of OKT®3 with Fc receptors on lymphocytes (Fc/FcR engagement).

Teplizumab is a 150-KD humanized mAb that binds the CD3-e epitope of the T cell receptor (TCR) complex with affinity equal to OKT®3, but it differs from OKT®3 in two properties:

1. The humanization process has resulted in the generation of a mAb that used less than 10% of the original murine amino acids in the antibody construction. The clinical consequence of this property is reduced immunogenicity or formation of anti-idiotypic antibodies.

2. Two amino acids have been changed (leucine$_{234}$ to an alanine and leucine $_{235}$ to an alanine) in the Fc portion of the immunoglobulin that disrupt Fc receptor and complement component C1q

binding. These two amino acid changes were aimed at eliminating the majority of cytokine-mediated toxicity observed during infusions of OKT®3.

The modified Fc component of teplizumab minimizes the activating capacity of the antibody compared with unmodified murine OKT®3. Although the primary mechanism of action of the antibody involves binding the CD3 antigen target on T cells, subsequent mechanisms involved in the therapeutic effects are incompletely understood. These mechanisms of action appear to involve weak agonistic activity on T cells as well as the generation of regulatory cytokines and regulatory T cells leading to the development of tolerance (20; 21).

2.3. Clinical Studies

As of March 2009, over 500 subjects have been treated with teplizumab including over 450 individuals with T1DM. Other subjects who have received teplizumab include subjects undergoing renal allograft rejection, individuals receiving islet transplantation, and patients with psoriatic arthritis.

Two clinical trials testing safety and efficacy have been completed using teplizumab in participants with recent onset T1DM; Study 1 (a phase I/II trial); Study 2 (protocol ITN007AI [NDB01]), as well as one PK/safety study (protocol ITN017AI). In addition, four clinical studies to preserve beta cell function in those with T1DM are underway. These studies are described below. Further information about these studies and other clinical experience with teplizumab are in the Investigator's Brochure.

2.3.1. Study 1: A Phase I/II Trial

Study 1 was a randomized, controlled, phase I/II, three-center trial that enrolled a total of 43 participants and tested two dosing regimens with hOKT3γ1(Ala-Ala)(23; 30). The clinical efficacy outcome tested was change in C-peptide response to MMTT in treated as compared to control groups.

The results of these studies suggest that treatment with the anti-CD3 mAb hOKT3γ1 (Ala-Ala) reduces the loss of insulin production over the first year in individuals with T1DM (Table 1)(23; 30).

***Table 1**. Changes in C-peptide response to an MMTT among participants in study 1*

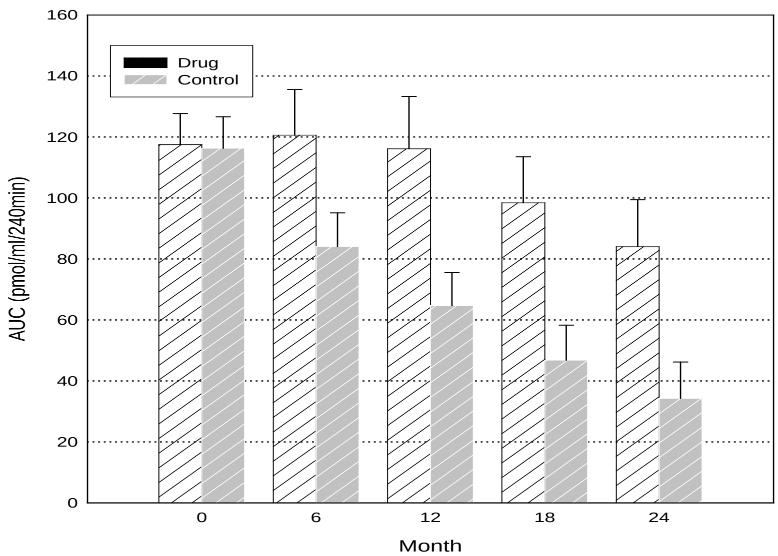
Patient Group	**Change at 6 months**			**Change at 1 year**		
	⇧	**No change**	⇩	⇧	**No change**	⇩
Drug treated	9	6	6	7	8	6
Control*	2	2	15	1	3	15

p<0.01; * Two control participants withdrew from the study.

At 1 year, the mean area under the curve (AUC) of the C-peptide response was 97±9.6% of the response at baseline in the drug-treated group (vs. 53±7.6% in the control group, p=0.001).

Follow-up was extended for 2 years after study entry. There continued to be a significant drug treatment effect at 2 years (p=0.002), although the meal stimulated C-peptide responses were falling in both the drug treated and control groups. The differences between the two groups were 44% at both year 1 and year 2 (31).

***Figure 2**. C-peptide response to an MMTT over 2-year period in study 1. There was a significant effect of drug treatment on the C-peptide responses over the 2 year period (p=0.002). (The data represent mean ± SEM of the study groups.)*



In the patients with diabetes, the drug treatment was associated with improvement in the hemoglobin A_{1C} (HbA_{1C}) levels (p=0.004) and reduced use of insulin (p=0.001) over the 2-year period.

2.3.2. Study 2: A Phase II Multiple-Dose Trial, NCT00806572

Protocol ITN007AI [NDB01]) started in June 2002 to assess the ability of the regimen to prolong the duration of the clinical response and to increase the number of responders (31). In study 2, the drug was to be administered soon after clinical diagnosis and at 6 and 12 months after diagnosis. Between June and August 2002, 10 patients were enrolled in study 2 (6 were randomized to the drug treatment group and 4 to the control group) and those assigned to the drug treatment group received one course of hOKT3γ1 (Ala-Ala) treatment. Further enrollment and hOKT3γ1 (Ala-Ala) treatments were stopped based on adverse event findings. Further investigation into the potential differences of the drug products and their preparations indicated that the absolute amount of drug that was administered to the six experimental patients in study 2 was greater than had been used in study 1.

2.3.3. ITN 017: A Phase I dosing study

Following discontinuation of Protocol ITN007, protocol ITN017 was conducted to determine a safe dose to be used for studies of teplizumab in subjects with Type 1 diabetes. Six subjects were enrolled in this open label trial. There were no serious adverse events in the first dosing cohort of 4 subjects. The study was discontinued when the first subject in the higher dosing cohort developed hyperbilirubinemia. The current dosing regimen is based on the findings from ITN017.

2.3.4. "ABATE Trial" ITN027 NCT00129259

This Phase II randomized open label study will test whether two courses of treatment (each for 14 days) within 8 weeks of diagnosis and at 1 year will improve C-peptide responses 2 years after diagnosis. There have been 6 serious adverse events in this trial. Three of these were judged to be study drug related. Two of the 3 were lymphopenia that was not unexpected, and one was cytokine release syndrome. This ongoing study completed enrollment in April 2009 (n=83).

2.3.5. "Delay Trial" NCT00378508

This randomized, double blind, placebo controlled trial will test the ability of a single 14 day course of treatment with teplizumab to prevent the loss of C-peptide 1 year after study enrollment. The "Delay Trial" offers optional retreatment for all subjects who retain detectable levels of insulin production, 1 year after study entry. The inclusion criteria for the Delay Trial differs from other studies in its enrollment of individuals with Type 1 diabetes of duration of 4 – 12 months. There have been two serious adverse events in this trial, neither of which was judged to be study drug related. As of March 2010, 50subjects have been enrolled with a planned enrollment of 60.

2.3.6. "Protégé Trial" NCT00385697

This is a Phase II/III multicenter study to compare efficacy, safety, and tolerability of 3 dose levels of teplizumab relative to placebo, in subjects within 12 weeks of T1DM diagnosis. Segment 1 was an open label study of 30 subjects which has completed enrollment. The double-masked, placebo controlled, four arm, trial is underway. The primary endpoint of the trial is an index that reflects hemoglobin A1c and insulin usage 12 months after study enrollment. Enrollment in this ongoing trial was completed in June 2009.

2.3.7. "Protégé Encore Trial" NCT00385697

This is a Phase II/III multicenter study to compare efficacy, safety, and tolerability of 3 dose levels of teplizumab relative to placebo, in subjects within 12 weeks of T1DM diagnosis. The double-masked, placebo controlled, four arm trial with a planned enrollment of 400 subjects is underway. The assessment of the primary endpoints is at Week 52. The study will continue to Week 104, with investigators and subjects remaining blinded to treatment assignment. The primary endpoint of the trial is an index that reflects hemoglobin A1c and insulin usage 12 months after study enrollment. Subjects will be dosed at randomization and approximately 6 months later.

2.4 Evaluations of Safety Experience with Teplizumab and the Basis for the Proposed Clinical Protocol

Safety reviews of the use of teplizumab in patients with type 1 diabetes were carried out in 2004, 2006, and continue on an ongoing basis by Data Safety and Monitoring Boards. These evaluations identified the occurrence of mild cytokine release, during the first 6 days of drug treatment, in about 10% of subjects. The 2006 safety evaluation noted that study drug was discontinued in 6/60 individuals due to adverse events characterized by laboratory abnormalities of consumptive coagulopathy and were thought to be due to the release of cytokines that occurred with the initial doses of drug. Specifically, the signs that identified this complex included any of the following: a) fever (of grade 3), b) an increase in the PT and/or PTT and an increase in the level of D-dimers, c) an increase in the total bilirubin level, d) hypotension, and e) an increase in liver enzymes. In each case, the abnormal findings began within the first 6 days of drug administration, and frequently with the first dose of drug. In all 6 cases, drug administration was stopped prior to administration to a complete course of protocol-defined treatment. The laboratory abnormalities were self limited, did not progress, and completely resolved without sequelae.

Based on these analyses, additional laboratory studies to evaluate the presence of cytokine release were recommended, and more stringent criteria for withholding drug were developed. With the modified criteria, all subjects who discontinued drug administration because of adverse events were identified by the second dose of drug. These new evaluations are incorporated into this and other ongoing protocols. More information is available in the Investigator's Brochure.

2.5. Use of Teplizumab in Children

The majority of new cases of type 1 diabetes occur in children under the age of 18. Moreover, given that the duration of diabetes is a significant risk factor for the development of diabetes complications and the clearly recognized difficulty in attaining excellent metabolic control of diabetes during adolescence (3; 32), it is critical that children are included in studies of the prevention of type 1 diabetes. The experience gained from DPT-1 and other studies demonstrates that children are more likely to have risk factors associated with rapid progression of the disease, suggesting a difference in the natural history of the disease from that in adults, and again demonstrating the need to include children in prevention studies.

600 subjects have participated in clinical trials of teplizumab: 100 in open-label studies (all treated with teplizumab) and approximately 500 in randomized, blinded studies (approximately 80% treated with teplizumab). See **Table 2** shown below which displays enrollment by age group.

"

Table 2: Age Distribution of Subjects by Study in Previous and Ongoing T1DM Studies (Data Available as of July 2009)

	Open-label (Treated with Teplizumab)						Blinded		Open-Label & Blinded
Age Range (years)	**Herold Study 1**	**ITN 007 (NDB01)**	**ITN017**	**ITN027 (Abate)**	**Protégé Segment 1**	**Total**	**Delay**	**Protégé Segment 2**	**Total**
8-11	9	2	2	24	11	**48**	7	73	**128**
12-17	8	1	4	23	16	**52**	9	189	**250**
≥18	5	3	0	4	11	**23**	2	230	**255**
Total	**22**	**6**	**6**	**51**	**38**	**123**	**18**	**492**	**633**

Notes: Protégé is Protocol CP-MGA031-01. The Herold studies are investigator-initiated studies. For further information regarding these studies, please refer to the Investigator's Brochure

Dr. Herold and colleagues reviewed the adverse event experience of the completed trials and concluded that although the number of subjects was small, there was no apparent relationship of age to the number or severity of adverse events.

In addition to the possibility of delaying or preventing the onset of T1DM, which is unproven, there are other prospects for direct benefit to children by their participation in the study. These include the recognized benefits of being in a clinical study, and of close monitoring for development of diabetes which significantly prevents morbidity associated with onset of the disease in the community including ICU admissions. The intervention has the prospect of direct benefit to the individual subject and in addition, is likely to yield general knowledge about T1DM which is of importance for the understanding and amelioration of T1DM in children.

The study procedures, while greater than minimal risk, offers the possibility of benefit due to the close monitoring for all participants, including children. Assent of children along with consent of the parents will be obtained prior to any study procedures. This research proposal in children is therefore consistent with United States Department of Health and Human Services, Protection of Human Subjects, 45CFR46.405 (research involving greater than minimal risk but presenting the prospect of direct benefit to individual subjects) and with 21CFR50.52 (Clinical investigations involving greater than minimal risk but presenting the prospect of direct benefit to individual subjects).

2.6. Additional information

Please refer to the Teplizumab Investigator's Brochure for further non-clinical and clinical information on the antibody.

3. STUDY DESIGN

3.1. Overview

This is a multicenter, double masked, randomized, placebo- controlled study to determine whether treatment of subjects at high risk for diabetes with teplizumab results in delay or prevention of clinical T1DM.

3.2. Objectives

3.2.1. Primary Objective

The primary objective of the study is to determine whether treatment of at-risk subjects with teplizumab results in a delay or prevention of T1DM.

3.2.2. Secondary Objectives

- to determine whether treatment with teplizumab is superior to placebo with respect to C-peptide responses to oral glucose, as obtained from timed collections during longitudinal tests
- to compare the safety and tolerability of teplizumab to placebo
- to assess the effects of treatment on mechanistic outcomes

3.3. Summary of Inclusion/Exclusion Criteria

Participants must meet all entry criteria for the protocol as outlined below.

3.3.1. Inclusion Criteria:

Study subjects must be or have:

1. Between the ages of 8-45 years.
2. A relative of a proband** with T1DM. If the proband is a parent, sibling, or offspring, the study participant must be 8-45 years of age. If the proband is a second or third degree relative (i.e. Niece, Nephew, Aunt, Uncle, Grandchild, Cousin), the study participant must be 8-20 years of age.
3. Subject (or parent or legal guardian) is willing to sign Informed Consent Form.
4. An abnormal glucose tolerance by OGTT confirmed within 7 weeks of baseline (visit 0)
    a. Fasting plasma glucose ≥ 110 mg/dL, and < 126 mg/dl*
    or
    b. 2 hour plasma glucose ≥ 140 mg/dL, and < 200 mg/dl
    or
    c. 30, 60, or 90 minute value on OGTT ≥200mg/dl
5. At least two diabetes related autoantibodies confirmed to be present on two occasions. The

autoantibodies that will be confirmed are anti-GAD65, anti-ICA512, anti-insulin (MIAA), and ICA. Confirmation of 2 positive autoantibodies must occur within the previous six months but the confirmation does not have to involve the same 2 autoantibodies.

6. Weigh at least 26 kg at randomization.

7. If participant is female with reproductive potential, she must have a negative pregnancy test on Day 0 and be willing to avoid pregnancy for at least one year from randomization.

8. If participant is male, he must be willing to avoid pregnancy in any partners for at least one year from randomization.

9. Willing and medically acceptable to postpone live vaccine immunizations for one year after treatment.

10. Willing to forego other forms of experimental treatment during the study.

*Fasting glucose levels of 110-126 qualify subjects as having abnormal glucose tolerance in this protocol as it reflects the criteria used for entry into the DPT-1 (33) and the DPT-1 data was used for the calculation of diabetes risk for this trial. Using data for individuals with Type 2 diabetes, the ADA uses a different glucose range to define impaired fasting glucose(34).

** A proband is an individual diagnosed with diabetes before age 40 and started on insulin therapy within one year of diagnosis. Subjects with probands who are considered to have type 1 diabetes by their physician but who do not meet this definition will be referred to the TrialNet Eligibility Committee for consideration for enrollment.

3.3.2. Exclusion Criteria:

Study subjects cannot have:

1. Diabetes, or have a screening OGTT with:
    a. Fasting plasma glucose ≥ 126 mg/dL, or
    b. 2 hour plasma glucose ≥ 200 mg/dL
2. Lymphopenia (< 1000 lymphocytes/μL).
3. Neutropenia (< 1500 PMN/μL).
4. Thrombocytopenia (< 150,000 platelets/μL).
5. Anemia (Hgb < 10 grams/deciliter [g/dL]).
6. Total bilirubin >1.5 x upper limit of normal (ULN).
7. AST or ALT >1.5 x ULN.
8. INR > 0.1 above the upper limit of normal at the participating center's laboratory.
9. Chronic active infection other than localized skin infections.
10. A positive PPD test.
11. Vaccination with a live virus within 8 weeks of randomization
12. Vaccination with a killed virus within 4 weeks of randomization.
13. A history of infectious mononucleosis within the 3 months prior to enrollment.
14. Laboratory or clinical evidence of acute infection with EBV or CMV.
15. Serological evidence of current or past HIV, Hepatitis B or Hepatitis C infection.
16. Be currently pregnant or lactating, or anticipate getting pregnant.
17. Chronic use of steroids or other immunosuppressive agents.
18. A history of asthma or atopic disease requiring chronic treatment.
19. Untreated hypothyroidism or active Graves' disease at randomization.
20. Current use of non-insulin pharmaceuticals that affect glycemic control.
21. Prior OKT®3 or other anti-CD3 treatment.
22. Administration of a monoclonal antibody within the year before randomization.
23. Participation in any type of therapeutic drug or vaccine clinical trial within the 12 weeks before randomization.
24. Any condition that, in the opinion of the investigator, would interfere with the study conduct or the safety of the subject.

3.4. Enrollment

Potential study subjects will be identified through the ongoing TrialNet Natural History study. In this study, first and second degree relatives of patients with T1DM are screened for biochemical autoantibodies and ICA. Those individuals who test positive are then further staged with the performance of an OGTT. The results of this OGTT will be used to determine eligibility for this protocol.

The TrialNet Natural History study screens participants at multiple clinical sites. The infusion of teplizumab or placebo will occur at a limited number of designated TrialNet treatment sites, whereas the initial visit and follow-up testing, as described in the Schedule of Events, may occur at other TrialNet sites.

3.5. Double-Masking and Description of Treatment Groups

The intervention will be conducted only amongst those who consent to participate. Subjects will be randomized to receive either teplizumab or placebo. All subjects will undergo close monitoring for the development of diabetes. Subjects and clinical investigators will be masked as to treatment assignment. The intervention protocol will be conducted at approved TrialNet clinical sites with appropriate facilities. All blood and serum samples for the primary and secondary outcome determinations will be sent to the Core Laboratories for analysis. Clinical laboratory studies that will be used for determining eligibility for study drug infusion will be done at the local sites.

Participants will be randomly assigned in a 1:1 ratio (within the two strata defined by age at enrollment: <18 and 18 or older) to the following 2 groups:

- to receive teplizumab (14-day IV infusion) followed by close monitoring for diabetes development
- to receive placebo (14 day IV infusion) followed by close monitoring for diabetes development

3.6. Treatment Assignment

After participants sign the consent form, complete the screening visit(s), and meet all of the inclusion criteria and none of the exclusion criteria, participants will be randomized to receive either teplizumab or placebo.

Participants will be randomized in equal allocations to each group. The randomization method will be stratified by TrialNet study site and whether the participant is less than 18 years of age or 18 years and older. This approach ensures that study site will not be a potential confounder. The TNCC will generate the randomization numbers and tables.

3.6.1. Procedures for Unmasking

Emergency unmasking will occur upon notification of the TrialNet Central Pharmacy and TrialNet Coordinating Center via the 24 hour emergency number and approval by TrialNet Chair, NIDDK TrialNet program officer, or TrialNet Medical Monitor. Non-emergent unmasking will occur upon notification of the TrialNet Coordinating Center and approval by TrialNet Chair or NIDDK TrialNet program officer. If unmasking is approved, the study sponsor and appropriate TrialNet committees (e.g. Safety Monitoring) will be notified of the event as soon as reasonably possible; however, they will not be unmasked.

3.7. Study Assessments

During the course of the study, participants will frequently undergo assessments of their glucose tolerance status, insulin production, immunologic status, and overall health and well-being (see Schedule of Assessments).

Samples will be drawn for storage in the National Institute for Diabetes and Digestive and Kidney Disease (NIDDK) Repository and at TrialNet Sites for future analysis.

3.8. Quality Assurance

During the study, duplicate collections of blood samples for assays will be obtained in a small sample of subjects for the purpose of external quality surveillance of the performance of the TrialNet central laboratories.

3.9. Study Timeline

3.9.1. Staggered Enrollment

Enrollment will initially be limited to subjects ages 16-45. Data from 10 randomized subjects through month 3 of the study (study visit 16) will then be evaluated by the DSMB and/or FDA or other applicable regulatory authorities to assess whether the observed adverse events in this group are consistent with adverse events seen in adult subjects with type 1 diabetes in previous studies. If there are no concerns, enrollment will then be open to individuals 12-45. The DSMB and/or FDA and other applicable regulatory authorities will then review adverse events after 10 subjects are treated and through month 3 of the study in this age group. If there are no concerns, enrollment will then be open to ages 8-45.

3.9.2. Study Duration

The study is designed to provide 80% statistical power to detect a 50% risk reduction with a one-sided test at the 0.025 significance level. This risk reduction is expected to result in a delay in the median time to onset of diabetes of 2.81 and 4.24 yrs. for the age cohorts < 18 and ≥18, respectively. To attain these design parameters will require the observation of 69 participants that are diagnosed on-study with T1DM. Consequently, the total sample size and study duration can only be approximated. The study plans to enroll approximately 140-170 subjects over approximately 2-3 years, and is projected to last between 4 and 6 years. As the study progresses, projections of the study end will be computed and updated based on the rate of enrollment, the observed hazard rate and the rate of loss-to-follow-up.

3.9.3. Follow-up Studies

Although subjects who develop diabetes will have reached the study endpoint, these individuals will be offered annual follow up for a minimum of two years Those individuals who have not developed diabetes by study end will continued to be followed as part of the TrialNet Natural History study protocol.

Individuals who develop T1DM may be eligible for interventional studies sponsored by TrialNet or other organizations under separate INDs. In the event that a subject wishes to participate in another investigational study that has, as an exclusion, treatment with experimental or immune modulatory drugs, the subject may request and be told of their treatment group assignment for the anti-CD3 prevention study. Every attempt will be made to minimize potential bias that this may introduce. The TNCC will make treatment assignment information available to the site investigator of the new study after the subject is determined to be willing to participate and not otherwise excluded from the new study. Other study group members will not be informed of the treatment assignment information. Mitigation of bias issues must be balanced against safety and interests of participants.

4. PATIENT MANAGEMENT

4.1. Screening Visit and Eligibility Assessment

This study will draw participants from the TrialNet Natural History Study.

The initial testing for autoantibodies, HLA type, and Oral Glucose Tolerance Test (OGTT) will be done as part of Natural History screening. Those individuals with two confirmed diabetes related autoantibodies and confirmed abnormal glucose tolerance on the OGTT, will then be eligible for additional tests and possible enrollment into Anti-CD3 Prevention trial.

Appendix 1 summarizes the flow of subjects from the Natural History Study into the Anti-CD3 Prevention Trial.

4.2. Anti-CD3 mAb Trial for At-risk Subjects Initial Visit

Prior to the initial visit, the Anti-CD3 Prevention Trial will be described to the potential participant. The participant/parent/guardian will be asked to sign an informed consent document describing the purpose, risks, and benefits of screening for the trial. A participant's signature indicates that he/she understands the potential risks and benefits of study participation. During these visits, a confirmatory OGTT and other clinical tests will be done to determine eligibility.

Any participant either not eligible or not willing to be randomized into the Anti-CD3 Prevention Trial is eligible for continued follow-up as part of the TrialNet Natural History Study.

4.3. Randomization and Baseline visits

Review will be made to be certain the subject meets study eligibility criteria. Prior to randomization, the intervention and follow-up parts of the study will be described to the participant. The participant/parent/guardian will be asked to sign an informed consent document indicating that he/she understands the study as well as the potential risks and benefits of study participation.

Participants will be randomized to either the treatment arm or the control arm. The randomization and the baseline visit must occur within 7 weeks of the confirmatory OGTT in order to ensure that participants have abnormal glucose tolerance at time of randomization and study drug administration. Note, subjects who are febrile at the time of baseline visit, may have the visit postponed up to five days outside the 7 week window if needed because of intercurrent illness.

4.4. Close Monitoring

During the study period, all participants will receive close monitoring for development of diabetes. OGTT tests will be performed at 3 and 6 months and every 6 months thereafter. In addition, at three month intervals in which there is no OGTT scheduled, a random (post-prandial) glucose level will be measured. At each visit and via interim visit phone calls, subjects will be asked directed questions about the presence or absence of symptoms associated with diabetes such as blurry vision, unintended weight loss of more than 3 kg, polyuria, and polydypsia. If subjects respond affirmatively to any of these questions or if any of the post-prandial glucose values are greater than 200 mg/dL, further evaluation, including fasting glucose or an OGTT, will be performed. Individuals in both of the study arms will have laboratory and mechanistic studies performed as detailed in the Schedule of Assessments.

4.5. Administration of Teplizumab

4.5.1. Drug Administration

Teplizumab or saline will be given by IV infusion over 14 days. The 14 day course must commence by 7 weeks after confirmatory OGTT, except in the case of interim illness as noted above. The dosing schedule is: Day 0: 51 mcg/m^2; Day 1: 103 mcg/m^2; Day 2: 207 mcg/m^2; Day 3: 413 mcg/m^2; Days 4-13: 826 mcg/m^2.

Teplizumab or saline is given as an IV infusion over a minimum of 30 minutes in the research or hospital setting. The infusion should be given at the same time each day +/- 4 hours. Vital signs will be monitored for 2 hours after each infusion. Subjects may leave the research or hospital setting each day upon completion of the 2 hours of post-infusion monitoring if they remain within approximately one hour of the treatment center.

The formulation of teplizumab will consist of:

- 10 mM sodium phosphate
- 150 mM sodium chloride
- 0.05 mg/mL Tween 80
- pH 6.1

Final drug product will be provided at a concentration of 1 mg/mL for a total of 2 mg of recoverable drug product per vial.

The vials should be stored upright at 2°–8° C and must not be frozen. Because there is no preservative and drug loss occurs over time, administration of study drug should begin as soon as possible and no later than 2 hours after preparation. The infusion must be complete within 6 hours of preparation. The drug may be prepared into a bag for infusion or into a syringe for delivery by infusion pump. Intravenous drug delivery devices, including IV bags and tubing, must be composed of PVC.

Laboratory studies that will be obtained prior to each dose are described in the Schedule of Assessments. The results of chemistries including liver function tests, WBC, Hgb, Hct, platelets, and INRs must be reviewed each day they are drawn prior to commencement of the drug infusion.

4.5.2. Drug Withholding in an Individual Subject During the 14 Day Treatment Period

Chemistries, liver function studies, CBC and differentials, and INR studies will be evaluated before drug is administered on each day that these studies are drawn as described in the attached Schedule of Assessments.

The following situations, laboratory abnormalities, or adverse events will lead to withholding of drug treatment *during the treatment course*: (Note: Day 0 is the first day of infusion)

1. Withdrawal of consent

2. Pregnancy for a female subject
3. Anaphylaxis requiring hemodynamic support (i.e., epinephrine and/or blood pressure medications) or mechanical ventilation.
4. Hepatic abnormalities*: Defined as total bilirubin >1.3 mg/dl on Day 1, ≥2.0 mg/dl on other days. AST level >2 times ULN on Day 1. AST, ALT or LDH ≥3.0 times ULN on other days.
5. Thrombocytopenia*: Defined as a platelet count < 140,000 on Day 1 and < 100,000 on other days.
6. Neutropenia*: Defined as <1000 cells/mm^3 (grade 3).
7. Anemia*: Defined as hemoglobin ≤ 8.5 g/dL or a drop in ≥2g/dL compared with prior to infusion to a value < 10.0 g/dL.
8. Coagulopathy*: INR > 0.1 above the upper limit of normal at the laboratory.
9. Fever: Grade 3 pyrexia on Day 0 or 1.
10. Other adverse events: Defined as a grade 3 or higher adverse event, regardless of relatedness to study drug, except for: lymphopenia, hypoglycemia, hyperglycemia, fatigue/malaise, insomnia, cheilitis, dry skin, nail changes, hot flushes/flashes, headache, myalgia, flu-like symptoms.
11. Any medically important event such as a concurrent illness, complications or abnormal laboratory test result that, in the opinion of the investigator, contraindicates continued dosing of study drug.

A laboratory test result meeting any of the above abnormalities noted by (*) should be confirmed on the same day as the initial test. Drug dosing will not occur while awaiting confirmation of the laboratory abnormality. If laboratory abnormalities are confirmed, or if any of the other situations listed above occur, the drug infusions will be discontinued in that patient and the procedures listed below will be followed. The drug infusions may not be resumed. Depending on the severity of the event, further reporting may be required as outlined below.

If the laboratory test is not confirmed when tested on the same day, drug dosing may be continued at the discretion of the investigator.

The Study Chair, TNCC, and Medical Monitor will be notified within 24 hours of any subject who is discontinued from study drug dosing. Subjects who are discontinued from teplizumab dosing will continue to receive follow-up care and evaluation as scheduled.

4.5.3. Further Evaluation after Withholding Infusions

The following are minimal assessments to be performed for those participants in whom an infusion is withheld (as described above):

1. LIVER TESTS: Total and direct bilirubin, AST, ALT, alkaline phosphatase the day the adverse event occurs and followed with frequent laboratory studies in order to establish the day that the event resolves. Additional studies may include an abdominal ultrasound to assess liver status and GI consult when necessary. If AST or ALT is > 3 x ULN and bilirubin >2 x ULN, evaluation should be done to determine if there is a cause other than study drug for these abnormalities (e.g., acute viral hepatitis, alcoholic and autoimmune hepatitis, biliary tract disorders, cardiovascular causes such as right heart failure or concomitant medications).
2. HEMATOLOGIC TESTS: CBC, differential, INR, D-dimer, and fibrinogen the day the adverse event occurs. The peripheral blood smear will be studied for evidence of RBC fragmentation. The CBC and differential and INR, will be frequently repeated in order to establish the day that the event resolves.

3. DRUG LEVELS AND SERUM CYTOKINES: Teplizumab levels and serum cytokines.
4. ADDITIONAL SAMPLES**:** Serum samples for storage and potential future analysis.

4.6. Interruption of Enrollment/Trial Cessation

Sections 4.5.2 and 4.5.3 describe monitoring and procedures for withholding drug treatment in individual patients. This section lists clinical and laboratory adverse events that will necessitate interruption of enrollment in the trial as a whole. As part of their ongoing safety review, the DSMB will make independent judgments regarding other adverse events requiring trial interruption.

1. Any drug related death*
2. Occurrence of anaphylaxis during study treatment in any participant. Anaphylaxis is defined in this protocol as a requirement of hemodynamic support or mechanical ventilation.
3. Stopping of drug infusions for criteria listed in section 4.5.2 in more than 2 of the first 10 enrolled subjects or in more than 20% of the total number of teplizumab treated subjects.
4. Grade 3 cytokine release syndrome (according to CTCAE criteria) at any time in more than 2 of the first 10 or more than 20% of the total number of teplizumab treated subjects.
5. The occurrence of ALT or AST >3x ULN and bilirubin >2x ULN at any time in any one subject.
6. Grade 3 hypotension at any time in more than 2 of the first 10 or more than 20% of the total number of teplizumab treated subjects.
7. Grade 3 thrombocytopenia at any time in more than 2 of the first 10 or more than 20% of the total number of teplizumab treated subjects.
8. Clinical mononucleosis syndrome which may include: Grade 2 or above fever, pharyngitis, lymphoadenopathy, splenomegaly, or rash, with detectable EBV viral load more than one week after the last dose of drug in any 3 of the first 10 or more than 25% of the total number of teplizumab treated subjects.
9. Severe adverse event: defined by CTCAE criteria of grade 3 or greater in any 2 of the first 3 patients or 3 of the first 7 drug treated patients at any time with the exception of Grade 3 lymphopenia within the first 30 days of drug treatment . In addition, severe adverse event of grade 3 or greater in more than 20% of the total number of teplizumab treated subjects.
10. Failure of the absolute number of lymphocytes to recover to 80% of the pretreatment level 2 months after the final dose of drug in 2 of the first 10 or 20% of the total number of teplizumab treated subjects.

In the event that these criteria are met, study enrollment will be suspended and the Institutional Review Boards/Ethics Committees/Research Ethics Board (IRB/EC/REB), and FDA and other applicable regulatory authorities will be notified that enrollment has been interrupted in order to perform a safety review of the enrolled subjects. The safety review will include a comprehensive evaluation of the safety experience from this trial as well as data from other ongoing studies with teplizumab in other disease settings. Before enrollment will resume, a satisfactory report of the safety review will be provided to the FDA, other applicable regulatory authorities, Institutional Review Boards/Ethics Committees/Research Ethics Boards (IRB/EC/REB), and the DSMB.

*During this trial, any death event will be temporarily considered unexpected and potentially drug-related until the event is adjudicated by the DSMB. In this event, the trial will be interrupted, including dosing of subjects already enrolled and enrollment of new subjects, until the death event is adjudicated by the DSMB and deemed “unlikely to be related to study drug.”

4.7. Prophylactic Medications

Ibuprofen and antihistamine will be administered prophylactically prior to teplizumab/placebo infusion on the first 5 days of treatment. Further dosing of Ibuprofen, antihistamines, and/or acetaminophen can be used as needed for fever, malaise, headache, arthralgia, or rash.

5. STUDY VISIT ASSESSMENTS

The schedule of evaluations and laboratory studies is presented in Appendix 2, Schedule of Assessments. A summary of assessments for the Protocol is given below.

5.1. General Assessments

General assessments for this Protocol will include:

- Informed consent
- Inclusion/exclusion criteria
- Medical history including lifestyle and participant experience assessment
- Physical examination including height/weight, abdominal circumference
- Concomitant medications
- Adverse events

5.2. Laboratory Assessments

The following clinical laboratory assessments will be performed during the study as described in the Schedule of Events (SOE):

- Chemistry (sodium, potassium, chloride, CO2, glucose, BUN, creatinine)
- Liver function tests (ALT, AST, LDH, alkaline phosphatase, total protein, albumin, total and direct bilirubin)
- Hematology (complete blood count with differential and platelets)
- INR
- Purified protein derivative (PPD) test
- Urine pregnancy test
- Antibodies to HIV, hepatitis B (antiHBcAb, HBsAg), hepatitis C (HCV),
- Cytomegalovirus antibodies (CMV IgG) and viral load
- Epstein-Barr virus antibodies (EBV IgG and IgM) and viral load
- Samples for virology and other immunization titers
- ECG

5.3. Mechanistic Outcome Assessments

TrialNet will perform immune and genetic assays to further understand mechanisms that may be underlying the type 1 diabetes disease process and response to therapy. For this purpose, samples for PBMC, DNA, RNA, plasma, and serum will be obtained. HLA testing may be done either under the auspices of TrialNet Natural History or this protocol.

5.4. Metabolic Outcome Assessments

Metabolic assessments will consist of:

1. OGTT
   - o Primary study outcome - Glucose tolerance status. The diagnostic criteria for diabetes from the 2003 Report of the Expert Committee on the diagnosis and classification of diabetes will be used (34). This study will be performed every 6 months or more frequently if clinically indicated based on a random glucose level of $\geq$ 200 mg/dl.
   - o The C-peptide and insulin data from the OGTT will be used to measure insulin secretion.
   - o The insulin, glucose and C-peptide data from the OGTT will be used to measure insulin sensitivity.

2. HbA1c
   - o Measure of glycemic control.

5.5. Laboratory Measures Related to Teplizumab Administration

Laboratory tests to measure drug level and immune response to the drug:

- Trough drug levels of teplizumab will be measured during the last 4 days of mAb administration in 12 subjects of each of the age strata: ≥ 16 yrs, 12-15 yrs, and 8-11 yrs.
- Antibodies against teplizumab will be measured at month 3 in 12 subjects from each age strata: ≥ 16 yrs, 12-15 yrs, and 8-11 yrs.

5.6. Visit Windows

The baseline visit must occur within 7 weeks after confirmatory OGTT (with the exception that individuals who are febrile at the time of the scheduled baseline visit, may have up to an additional 5 days). Visit 14 must be +/- 2 days. Visit 15 is to be +/- 4 days. All other visits described in the Schedule of Events can be ± 3 weeks.

6. ADVERSE EVENT REPORTING AND SAFETY MONITORING

6.1. Adverse Event Definition

6.1.1. Adverse Event

In this clinical trial, an adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom or disease whether or not associated with the treatment and study procedures.

Throughout the study, the investigator must record all adverse events on source documents. Events not related to diabetes onset, hypoglycemia, or hyperglycemia which are Grade 2 or greater per the NCI CTCAE (see Section 6.1.4. Grading Event Severity below) must be reported to TNCC on the appropriate Adverse Event form. The investigator should treat participants with adverse events appropriately and observe them at suitable intervals until the events resolve or stabilize.

Adverse events may be discovered through:

- observation of the participant;
- questioning the participant;
- unsolicited complaint by the participant

Questioning of the participant should be conducted in an objective manner.

6.1.2. Serious Adverse Event

A serious adverse event (SAE) or reaction is defined as “any adverse event occurring at any dose that suggests a significant hazard, contraindication, side effect, or precaution.” This includes but is not limited to any of the following events:

1. Death. A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up after the completion of therapy must be reported whether it is considered to be treatment related or not.
2. A life-threatening event. A life-threatening event is any adverse therapy experience that, in the view of the investigator, places the participant at immediate risk of death from the reaction as it occurred.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant disability.
5. An event that required intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
6. Congenital anomaly or birth defect.
7. Grade 4 or higher lymphopenia for 7 or more days occurring in the first 30 days after the start of the teplizumab infusion.

8. Grade 3 or higher lymphopenia occurring anytime later than the first 30 days after the start of the teplizumab infusion.

Regardless of the relationship of the adverse event to study drug, the event must be reported as a serious adverse event if it meets any of the above definitions.

6.1.3. Unexpected Adverse Event

An adverse event is considered unexpected when the nature (specificity) or severity of the event is not consistent with the risks described in the Investigator's Brochure or the informed consent document.

6.1.4. Grading Event Severity and Causality

TrialNet has adopted usage of the National Cancer Institute (NCI) Common Technology Criteria for Adverse Events (CTCAE) and/or study-specific criteria for classification to describe the severity of adverse events. Hypoglycemia and hyperglycemia will be reported as adverse events only in the case of requiring the assistance of others due to loss of consciousness or DKA. TN Investigators will also provide an assessment of relationship of AE to study drug as not, unlikely, possibly, probably, or definitely related.

6.2. Adverse Event Reporting and Monitoring

Adverse events will be reported to the TrialNet Coordinating Center. The investigator will grade their severity according to common toxicity criteria or study-specific criteria and will make a determination of their relation to therapy. Events will be assessed and reported consistent with the ICH Guideline for Good Clinical Practice and per the guidance of the DHHS Office for Human Research Protections (OHRP).

The adverse event case report form for the protocol must be completed for all adverse events (AE). For reporting serious adverse events (SAE), the TrialNet MedWatch Form should also be completed and faxed to the TNCC *within 24 hours of when the site was notified of the event.* This will be reviewed by the TrialNet Medical Monitor, the TrialNet Safety Committee, and the DSMB as appropriate. Deaths must be reported immediately. Event outcome and other follow-up information regarding the treatment and resolution of the event will be obtained and reported when available, if not known at the time the event is initially reported. The follow-up information should contain sufficient detail to allow for a complete medical assessment of the case and an independent determination of possible causality.

Adverse events will be assessed by the TrialNet Medical Monitor. The DSMB will conduct regular safety reviews approximately every three to six months (and otherwise as needed) of adverse events by treatment group assignment. Serious adverse events as well as adverse events leading to study discontinuation will be reviewed by the DSMB. All adverse events will also be reported to MacroGenics by the TNCC.

7. PARTICIPANT SAFETY

7.1. Protecting Against or Minimizing Potential Treatment Risks

Subjects will not be enrolled who have other active serious medical problems. Frequent monitoring of patients with history, physical examination, and laboratory studies will allow for early identification of adverse events. All participants will be required to have adequate hemoglobin to allow safe frequent venipuncture. Every attempt will be made to minimize the number of venipunctures.

All infusions will take place in a facility that has resuscitation capabilities.

Participants will be counseled by study personnel and requested to avoid pregnancy for 1 year following drug administration for safety purposes. This applies to females on study and female sexual partners of males.

7.1.1. Prohibited Medications

Participants will be instructed not to use Prednisone, other immunosuppressive agents, or chronic inhaled or nasal corticosteroids during this trial in order to reduce infectious risks and to prevent possible impact on progression to diabetes. However, as an intention to treat study, no individual will be withdrawn from analysis if this occurs.

Participants who receive teplizumab/placebo will be instructed not to receive live vaccinations for 1 year after dosing. In addition, participants should not receive vaccination with a killed virus vaccine less than 4 weeks after treatment with study drug unless approved by the study chair or the study ID team.

7.2. Expected Side Effects and Adverse Events

A full description of the adverse events experienced by subjects in trials using teplizumab is in the Investigator's Brochure. The descriptions below highlight the most common drug related events and potential adverse events.

7.2.1. Hematologic

The drug causes a reduction in the number of circulating lymphocytes. Grade 3 or higher lymphopenia has been seen during drug administration in 15% of subjects. However, in more than 85% of individuals, circulating lymphocytes return to ≥80% of baseline values by 2 months after initiation of treatment. A single SAE (i.e., prolonged CD4 cytopenia) has occurred in a patient who was given 2 times the proposed dose of drug. This patient did not develop infections and the CD4 cytopenia resolved spontaneously after two years.

Neutropenia and thrombocytopenia have also been seen during drug administration. Overall these adverse events have occurred in < 5% of subjects but have been up to grade 3 in < 2% of individuals. They have resolved spontaneously or with withholding of drug in all cases. This risk will be mitigated by having platelet and neutrophil count reviewed before administration of teplizumab/placebo as indicated in the SOE. Specific treatment stopping rules are described in Section 4.5.2.

Mild anemia has been seen in 21.9% of subjects. This risk will be mitigated by having the hemoglobin reviewed before administration of teplizumab/placebo as indicated in the SOE.

7.2.2. Cytokine Release Syndrome

Cytokine release syndrome (CRS) has been described in 5.7% of drug treated subjects – the syndrome was mild or moderate in 5/6 reported subjects. Compared to FcR binding anti-CD3 antibodies like OKT3, the CRS that has occurred with Teplizumab is reduced in frequency and severity. The clinical experience to date suggests that the occurrence of CRS may be seen with the initial doses of the drug and is dose related. The single reported case of moderate disseminated intravascular coagulation was related to the occurrence of cytokine release syndrome. In a previous phase II trial (using a drug dose that is 2 times higher than the proposed dose), symptoms of CRS—including headache, nausea, vomiting, fever, myalgias, arthralgias, and shaking—occurred over the first 3 days of drug treatment, but subsequently resolved. The potential for occurrence of cytokine release syndrome has led to the drug withholding rules listed in Section 4.5.2.

Manifestations of CRS have also included hyperbilirubinemia and increased liver function tests. In the PK/safety trial (ITN017AI), a grade 4 direct hyperbilirubinemia, which may have been a manifestation of a cytokine release syndrome, was observed.

Transient increases in the alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) up to 5 times normal (grades 1 and 2) levels have been seen in all trials. These abnormalities have been transient. Grade 1 hypoalbuminemia has been seen in patients receiving the anti-CD3 mAb with other immunosuppressive agents for prevention of transplant rejection.

This risk will be mitigated by having INR and liver function tests, including bilirubin, reviewed before administration of teplizumab/placebo as described in the SOE and the specific drug withholding rules listed in Section 4.5.2.

7.2.3. Lymphoproliferative Disease

Although not raised as an issue in the single-dose studies of hOKT3γ1 (Ala-Ala), immunosuppression of any sort may predispose participants to additional risks such as infection or lymphoproliferative disease. On a theoretical basis, this risk is minimal since the total duration of immunosuppression is short. Clinical experience in transplant recipients, treated with other biologic agents, suggests that the risk of lymphoproliferative disease is highest in participants who develop infections with EBV around the time of immunosuppression. Nonetheless, the lymphoproliferative syndrome associated with reactivation of EBV infection that was seen in an islet transplant patient treated with teplizumab and other immunosuppressive drugs occurred in a subject who was EBV IgG+ before study entry. Therefore, a careful history will be taken regarding development of mononucleosis-like illnesses during the period preceding and after study enrollment. Subjects will be screened for detectable EBV and CMV viral loads – the finding of a positive viral load will preclude enrollment for at least 90 days after the viral load becomes undetectable. In this situation, the subject will need to repeat OGTT assessments to ensure continued eligibility for the trial. EBV and CMV viral will be measured after drug treatment as described in the SOE. This aggressive monitoring scheme will allow us to determine whether changes in lymphocyte subsets are associated with reactivation of latent viruses.

7.2.4. Anti-idiotype Responses

Anti-idiotype antibodies have been detected in up to 50% of patients administered teplizumab. The presence of these antibodies may diminish efficacy of future cycles of study drug and/or lead to manifestations of antigen-antibody complexes such as serum-sickness illness or hypersensitivity reactions. The titer of these anti-idiotype antibodies has been < 1:1000 and patients with anti-idiotype antibodies have been retreated with Teplizumab without adverse effects or detectable changes in the efficacy of the drug. To date, no adverse effects have been reported as a result of these antibodies.

7.2.5. Infection

As with any therapy that suppresses the immune system, there is a risk of developing infections. On a theoretical basis, this risk is minimal, as the total duration of immunosuppression is short. Overall, in open labeled trials with teplizumab, 49.5% of subjects have experienced infections of any kind. Of these, 48.6% were classified as mild or moderate. There have been two cases of TB reported in Protégé trial participants in India and Ukraine respectively. However, trial remains blinded so relatedness is not known at this time.

This risk will be mitigated by having subjects report even mild illness between study visits. They will be specifically asked about infectious adverse events during the study visit, and they will be monitored regularly for infections and appropriate anti-infective therapy will be instituted if indicated. Consultation with TrialNet infectious disease team will be available. All infectious adverse events will be reviewed by the TrialNet ID team, Medical Monitor and DSMB if serious.

7.2.6. Rash

Rash has been seen in 42-62% of patients treated with drug. The rashes that have been observed include a macular rash on the face, neck, and trunk, as well as a maculopapular rash on the extremities. The latter rash has occurred on the hands and feet and has resolved spontaneously but with peeling of the skin. Biopsies of the rash performed in two subjects showed histologic findings of spongiosis consistent with eczematous dermatitis. A severe rash occurred in one subject, receiving hOKT3γ1(Ala-Ala) for prevention of islet allograft rejection on the 3rd day of drug administration. It was classified as severe and the patient was hospitalized. A biopsy of the rash showed a moderate mixed perivascular dermal infiltrate consistent with a drug reaction. Supportive care was given, the drug dosage was reduced, and the rash resolved.

7.3 Pregnancy

Pregnant and lactating women will not be included in the study. Females must have a negative pregnancy test prior to enrolling in the study and will be required to use a reliable and effective form of birth control during the study. Male participants will also be required to prevent pregnancy in their partners. At every study visit the sexual activity of participants of reproductive age will be re-assessed. If a subject who was previously sexually inactive becomes sexually active, s/he will be counseled about the need to use a reliable and effective form of birth control. Female subjects will also be required to undergo urine pregnancy tests at regular intervals including prior to teplizumab/placebo administration. A positive pregnancy test will result in holding of scheduled drug infusion.

All pregnancies that are identified during the study must be followed to conclusion and the outcome of each must be reported. The investigator should be informed immediately of any pregnancy whether occurring in a female participant or the female partner of a male participant. The investigator should report all pregnancies to TrialNet within the same timeframe (24 hours) as SAEs, using the SAE report form. Monitoring of the participant should continue until the conclusion of the pregnancy, and a follow-up SAE report form detailing the outcome of the pregnancy should be submitted to TrialNet.

8. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

Analyses of study data will be conducted to address all objectives of the trial and other interrelationships among data elements of interest to the investigators and of relevance to the objectives of the study. Analyses by gender and race/ethnicity, as appropriate, are also planned.

Primary analysis of treatment effect will be conducted under the intention-to-treat principle whereby outcome data from all subjects randomized will be included regardless of treatment compliance.

8.1 Primary Outcome

The primary outcome is the elapsed time from random treatment assignment to the development of diabetes or time of last contact among those randomized.

Criteria for diabetes onset (T1DM) are, based on glucose testing, or the presence of unequivocal hyperglycemia with acute metabolic decompensation (diabetic ketoacidosis). One of the following criteria must be met on two occasions as soon as possible but no less than one day apart for diabetes to be defined:

1. Symptoms of diabetes plus casual plasma glucose concentration ≥ 200 mg/dL (11.1 mmol/l). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.

2. Fasting plasma glucose ≥ 126 mg/dL (7 mmol/l). Fasting is defined as no caloric intake for at least 8 hours.

3. 2 hour plasma glucose ≥ 200 mg/dL (11.1 mmol/l). The test should be performed using a glucose load containing the equivalent of 1.75g/kg body weight to a maximum of 75 g anhydrous glucose dissolved in water.

It is preferred that at least one of the two testing occasions involve an OGTT.

Cases identified will be confirmed as having diabetes if the glucose values to make these determinations were obtained in a TrialNet laboratory as part of an OGTT. Cases diagnosed with diabetes by symptoms and casual glucose >200mg/dl or by other criteria than the above will be adjudicated by the TrialNet Diabetes Adjudication committee.

8.2 Primary Analysis

The study design is a randomized double-blind placebo controlled trial. The primary objective of the TrialNet Anti-CD3 Trial is to assess the effect of teplizumab versus control on the risk of diabetes onset in the target population as defined by the eligibility criteria.

The cumulative incidence of diabetes onset over time since randomization within each treatment group will be estimated using the Kaplan-Meier method (proportion surviving diabetes-free as a function of time). The difference between groups in the cumulative incidence functions, and the associated hazard functions, will be tested at the 0.025 level, one-sided, using the Cox Proportional Hazards (PH) model (35, 36). The estimates of cumulative incidence of diabetes and the test will be adjusted for age at enrollment. The relative risk of diabetes onset between groups will be estimated from the PH model. The critical value for the Wald test statistic, and confidence intervals, in this primary analysis will be determined by the group-sequential procedure outlined in the section entitled Interim Monitoring Plan below.

8.3 Secondary Outcomes and Analyses

A variety of secondary analyses are planned, some of which will include the following.

1. Subgroup analyses will be conducted comparing the effects of teplizumab versus control on the risk of diabetes within subsets of the study cohort, such as among men versus among women. Subgroups of the population will be classified by age (group cut-offs will be based partial on the available data) gender, race/ethnicity, specific antibody status at baseline, and presence or absence of HLA DQB1*0602. Differences in the treatment effect between subgroups will be tested using a covariate by group effect in a Cox PH model, including age as a quantitative covariate.

Similar analyses will be conducted using the values of quantitative baseline factors including age, weight, BMI, and the immunologic and metabolic factors described in Section 5 that include the autoantibody titers, basal C-peptide, OGTT stimulated C-peptide (peak and AUC mean), and measures of insulin resistance modeled from the OGTT. The dependence of the treatment effect on the quantitative levels of each factor will be assessed through a covariate by treatment group interaction in a PH model. Such an analysis will also be conducted to assess the effects of age as quantitative covariates.

Additional covariates may be defined during the conduct of the study. The reporting of the analyses will distinguish between factors specified prior to primary analysis and those identified post-hoc during analysis.

2. Longitudinal analyses will assess the effects of teplizumab versus control treatment on immunologic and metabolic markers over time up to the onset of diabetes. Differences between groups in the mean levels of quantitative factors over time will be assessed using a normal errors linear model for repeated measures. Differences between groups in the prevalence of qualitative factors over time will be assessed using generalized estimating equations for categorical measures. Generalized estimating equations may also be employed for the analysis of quantitative factors when the normal errors assumptions are violated (37).

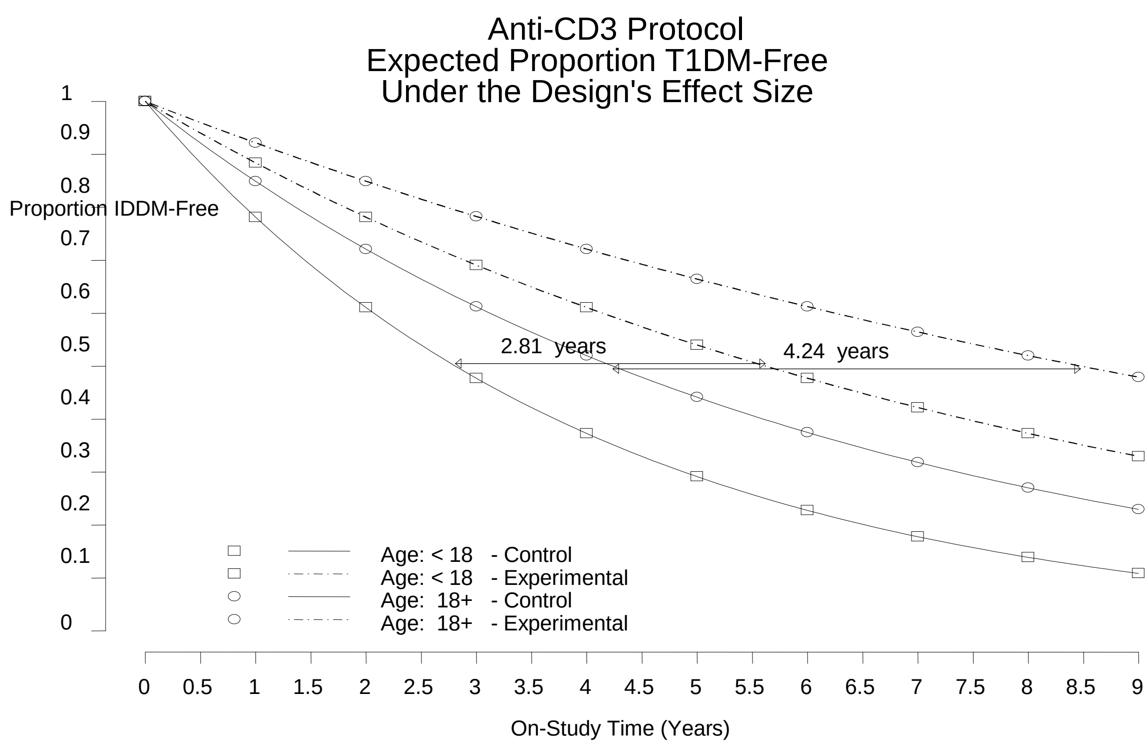
Once a subject develops diabetes, the subject will have reached the primary outcome of the study. However, the subject may still be followed for assessment of other outcomes that will permit continued longitudinal assessment of metabolic and immunologic parameters.

3. The association of demographic, genetic, immunologic, metabolic, and lifestyle factors, among others, both at baseline and over time, with the risk of diabetes onset will be assessed in Cox PH Models over time. The effects of changes in longitudinal factors on diabetes risk will be assessed using time-dependent covariates for these factors. Analyses will be conducted separately within the

treatment and control groups, and differences between groups in covariate effects (group by covariate interactions) will be assessed.

8.4 Study Power and Sample Size

Applying the eligibility criteria for this study to the data from the Natural History Study (TN-01), hazard rates were estimated for the control group assuming a constant risk over time. The maximum likelihood estimates (MLE's) were 0.247 and 0.164 per year for the age cohorts of 8 - 17 years and 18 - 45 years, respectively. Continuing with the assumption of a constant hazard rate, the median time to diabetes onset is 2.81 and 4.24 years for the age cohorts of 8 - 17 years and 18 - 45 years, respectively. This study has been designed to detect a 50% reduction in the risk of T1DM (i.e., effect size; usually expressed as a hazard ratio of experimental to control, of 0.50). In terms of the median time to T1DM, this effect size represents a 2.81 and 4.24 year delay among those treated with teplizumab (compared to controls) for the age cohorts of 8 - 17 years and 18 - 45 years, respectively. These design characteristics are displayed in the graph below. Based on the Natural History Study, 55% of the enrolled participants will be 8 - 17 years of age. Using this percentage, the weighted average hazard rate and median time to T1DM for the control group is projected to be 0.209 per year and 3.31 years, respectively.



To achieve statistical power of 80% for a one-sided Wald test at the 0.025 significance level and the effect size described above, will require enrollment and follow-up of enough participants to observe 69 T1DM cases (38) (this is the "event" sample size in contrast to the study sample size). This event sample size reflects the combination of the study sample size and the amount of follow-up at which the fixed-sample primary hypothesis test may be conducted. Although group sequential testing will be employed, the method of Lan and DeMets maintains the power while controlling the type I error used in determining the fixed sample size.

The study sample size and duration are variable when fixing the "event" sample size. In the absence of any safety concerns and evoking any stopping rules, closing accrual should not occur until sufficient participants are accrued so that projections (based on the observed T1DM rates and the actual accrual pattern) indicate that within a reasonable follow-up period the event sample size will be achieved. The constant hazard rate assumption is retained to compute the initial projection. It is anticipated there will be an initial group of subjects available for immediate enrollment followed by additional subjects accrued over the open enrollment period of the study. Based on the ongoing Natural History Study, there are 221 subjects known to be eligible. It is assumed that 88 (40%) of these subjects will consent to participate in the trial and will be available for immediate enrollment. Based on the Natural History Study the rate of new patients that would be eligible for this study is 66 per year. Again, assuming 40% will consent to participate yields a 26.4 per year accrual rate. Allowing for a 5% per year drop-out rate, the table below provides several accrual periods and overall study durations that will provide the advertised statistical power (39).

Table 3: Possible Accrual Periods and Overall Study Durations to Achieve the Event Sample Size
$(1-\beta = 0.80;\ \alpha(\text{1-tail}) = 0.025)$

Enrollment Period in Years (Total Study Sample Size)	Study Duration in Years (includes Enrollment and Follow-up Period)	The Minimum Number with T1DM at the End of the Trial
2.0 (142)	5.75	69
2.5 (155)	5.18	69
3.0 (168)	4.84	69

It should be noted that the shortest study duration is achieved by continuing accrual until the event sample size is reached (i.e., essentially no follow-up time for the last enrolled patient). However, this also requires the largest number of participants to be enrolled. Although somewhat arbitrary, our intent is to accrue from 2 to 3 years. Based on the research strategy at the time, a decision can be made to stop enrollment and start the follow-up period. This will be done in consultation with the TrialNet governing body as well as the DSMB.

Note the accrual period and the study sample size are only projections since the actual accrual rate, the control hazard rate and the loss to follow-up rate are unknown. Furthermore, the over-all hazard rate is sensitive to the age distribution of the study population which is also unknown. As the study progresses, more accurate projections of the study duration will be computed based on the observed data and will be provided to the DSMB and the TrialNet governing body, and if need be, this document will be amended.

8.5 Interim Monitoring Plan

Interim analyses will be conducted periodically during the study and will be reviewed by the TrialNet Data and Safety Monitoring Board (DSMB) for assessment of effectiveness and safety. The same primary endpoint analysis described above will be used to evaluate the evidence of a treatment group effect during interim analyses. The Wald test from the PH model will be transformed to a z-score (with negative values indicating a reduction in risk in the teplizumab treated group). If a group sequential stopping boundary is crossed, the DSMB may terminate the trial early. The Lan and DeMets (40) spending function with an O'Brien-Fleming boundary will be used to protect the type I error probability for the primary outcome analyses, and to assess the significance of the interim results periodically during the trial. The spending function (α_1) that approximates the O'Brien-Fleming boundaries is:

$$\alpha_1(t^*) = 2 - 2\Phi\left[\frac{Z_{\alpha/2}}{\sqrt{t^*}}\right]$$

where t^* is the information fraction ($0 < t^* \leq 1$) and α is the fixed-sample type I error (i.e., 0.025).

The DSMB will also be informed if there is a serious lack of evidence of a treatment effect (i.e. futility analysis). The boundaries are based on the paper by Lachin (41). The study should be stopped based on the futility of rejecting the null hypothesis at the completion of the trial if: $Z_{HR}(t^*) \geq 0$ when $0.5 \leq t^* < 0.8$ or if $Z_{HR}(t^*) \geq -0.8$ when $t^* \geq 0.8$. Lachin showed that a onetime use of either boundary contributes less than 0.003 to the type II error when t^* is equal to 0.5 and 0.8, respectively. It is straight forward to show that if conducted at a larger value of t^* the increase to the error probability is even less. Furthermore, a single use of each rule will increase the type II error no more than twice this probability (i.e., 2x0.003). Simulation studies conducted confirmed that this rule increases the type II error by 0.0029 (in 20,000 simulations).

8.6. Withdrawal Criteria- Individual Subjects

An intent–to-treat approach will be used. Subjects will not be replaced. All data acquired prior to termination for the reasons outlined below will be included in the primary analysis unless patient withdraws consent. Every effort will be made to conduct a final study visit with the participant and participants will be followed clinically until, if applicable, all adverse events resolve.

- Withdrawal of consent
- Withdrawal by the participant
- Withdrawal by the investigator
- Intercurrent illness or event that precludes further visits to the study site or ability to evaluate disease

9. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

9.1. Statement of Compliance

This study will be conducted in compliance with the protocol and consistent with current Good Clinical Practices (GCP), adopting the principles of the Declaration of Helsinki, and all applicable regulatory requirements (*ICH E6, 45CFR46, and FDA 21CFR sections 11, 50, 56, 312)*.

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate Independent Ethics Committee/Research Ethics Board (IEC/REB) or Institutional Review Board (IRB). Any amendments to the protocol or consent materials must also be approved before they are implemented.

9.2. Participating Centers

Participating TrialNet clinical sites must have an appropriate assurance, such as a Federal-wide Assurance (FWA) or an Unaffiliated Investigators Agreement (UIA), with the Office for Human Research Protections (OHRP), since they are actively engaged in research and provide informed consent. The protocol and consent forms will be approved by Institutional Review Boards or Ethics Committees/Research Ethics Boards at each of the participating clinical sites. HIPAA and applicable local regulations will be followed by each participating institution in accordance with each institution's requirements. The participating international sites will obtain approval from their corresponding review boards in accordance with their local procedures and institutional requirements.

The investigator is required to keep accurate records to ensure the conduct of the study is fully documented. The investigator is required to ensure that all case report forms are legibly completed for every participant entered in the trial.

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants participating in this study. When a subject participates in this study at more than one TrialNet site, sharing of this information is required. Medical and research records will be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational site must permit authorized representatives of the sponsor(s) and regulatory agencies to examine (and when required by applicable law, to copy) records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress. Unless required by the laws permitting copying of records, only the coded identity associated with documents or other participant data may be copied (obscuring any personally identifying information). Authorized representatives, as noted above, are bound to maintain the strict confidentiality of medical and research information that may be linked to identify individuals. The investigational site will normally be notified in advance of auditing visits.

9.3. Informed Consent

The process of assuring that individuals (and parent/guardian if less than 18 years of age) are making an informed decision about participating in this study includes both verbal and written communication. Written materials include a Patient Handbook and written consent forms. There are several consent forms for this study. One is a Screening consent form that describes the procedures, risks, and benefits, and determines eligibility for the study. The second is the Intervention consent form, which describes the procedures, risks, and benefits for the remainder of the study. A third consent form is for use at clinical sites that will be performing the post-treatment visits, but not the treatment visits. The consent forms will be reviewed with participants (and their guardian in the case of participants under 18 years of age) and the participant will be given time to review the written consent form and ask questions. An assent form has also been developed for participants less than 18 years of age (unless local IRB requirements differ in procedure).

As part of the informed consent process, the participant and/or parent or guardian (if the participant is less than 18 years of age) will also be required to complete a short, written Volunteer Understanding Quiz that is designed to ensure that the subject understands the study, as well as what is being asked of him/her. The participant will be given a copy of their signed consent/assent forms.

The consent process will be conducted by qualified study personnel (the Trial or Study Coordinator and/or Investigator or other designee). All participants (or their legally acceptable representative) must read, sign and date a consent form prior to participation in the study, and/or undergoing any study-specific procedures.

The informed consent form must be updated or revised whenever there is new, clinically significant information applicable to the safety of the participants, when indicated for a protocol amendment, and/or whenever any new information becomes available that may affect a patient's participation in the study.

Subjects will be re-consented if they reach the age of 18 years while enrolled in the study.

9.4. Study Subject Confidentiality

Study records with the study subject's information for internal use at the clinical sites will be secured at the study site during the study. At the end of the study, all records will continue to be kept in a secure location. There are no plans to destroy the records.

Study subject data, which is for reporting purposes, will be stored at The TrialNet Coordinating Center. Case report forms sent to the Coordinating Center will identify participants by the unique TrialNet Identification Number. The data entry system at the Coordinating Center is a secured, password protected computer system. At the end of the study, all study databases will be archived at the Coordinating Center, and the data collection forms will be electronically scanned and saved in electronic format for long-term storage. De-identified safety data will be shared with MacroGenics during the course of the study so the company may meet its regulatory reporting requirements as the drug manufacturer and IND sponsor. Additional de-identified data will be shared with MacroGenics at the end of the study.

Stored samples including genetic samples could be utilized to learn more about causes of type 1 diabetes, its complications (such as eye, nerve, and kidney damage) and other conditions for which individuals with diabetes are at increased risk, and how to improve treatment. The results of these future analyses, and any mechanistic studies will not be made known to the participant.

9.5. Risks and Benefits

The risks of this study are presented in this protocol, the Investigator's Brochure and informed consent form. There is no guaranteed benefit to subjects for their participation in the study. This study will examine whether intervention with teplizumab will delay or prevent the onset of diabetes, but there is no guarantee that this will occur. However, all subjects will benefit from close monitoring for the development of diabetes. This close monitoring significantly reduces the morbidity typically associated with clinical onset of disease.

Special consideration regarding risks and benefits for children is described in section 2.5.

9.6. Ethics

The study protocol, along with the required informed consent forms, will be approved by each participating institution's Institutional Review Board (IRB) or Ethics Committee/Research Ethics Board (EC/REB) at international sites prior to the initiation of any research procedures (at the site). In addition to details described in the sections above (informed consent, confidentiality, and risks and benefits) the investigators have reviewed and considered ethical ramifications in the design and development of this protocol. The investigators have made every effort to minimize and monitor risks and discomforts to participants throughout the course of the study.

10. STUDY ADMINISTRATION

10.1. Organizational Structure

This study is part of Type 1 Diabetes TrialNet, which is funded by the National Institutes of Health. Funding will cover the costs of administration and laboratory tests associated with this study during the participant's period of follow-up.

10.2. Role of Industry

The IND holder is MacroGenics, Inc. MacroGenics will provide teplizumab and placebo for the study and financial support for clinical trial monitoring supplemental to standard TrialNet procedures. Eli Lilly and Company holds an exclusive license from MacroGenics to develop and commercialize teplizumab. Under TrialNet's direction, MacroGenics will perform measurements such as PK and anti-teplizumab antibodies as indicated on coded samples. Data and data analysis will be conducted by TrialNet investigators.

10.3. Groups and Committees

10.3.1. Anti-CD3 Prevention Study Chair

The Study Chair and TrialNet executive committee will receive periodic reports from the TrialNet Coordinating Center on the progress of the study. These will include accrual rates and baseline demographic characteristics. Interim data summaries provided to others (except those that could lead to unmasking of the treatment arms) will first be supplied to the Study Chair for review. Criteria and results of ongoing monitoring of the TrialNet labs in terms of reproducibility will also be provided on a routine basis and reported on during Anti-CD3 Prevention Study Committee meetings, as scheduled. As appropriate, abstracts and manuscripts dealing with the progress of the trial shall be directed by the Study Committee.

10.3.2. TrialNet Chairman's Office and TrialNet Coordinating Center

The TrialNet Chairman's Office and TrialNet Coordinating Center (TNCC) will work together in providing leadership to the TrialNet study group to include protocol and manual preparation, training for clinical sites, development of statistical design for each study, and analysis of study results. The TNCC will also coordinate interactions among the participating TrialNet clinical centers, test laboratories including TrialNet core laboratories and other subcontract laboratories, NIDDK, and other sponsoring agencies.

10.3.3. Clinical Sites

Each Principal Investigator at the participating TrialNet clinical site will oversee all operations at that site. The clinical sites will forward all laboratory and data collection form information to The TrialNet Coordinating Center for analysis. Conference calls and site visits, as needed, will facilitate evaluation of the trial management. Certain TrialNet sites will be involved in recruitment and follow up of subjects and some sites will also administer study drug.

10.3.4. Diabetes Adjudication Committee

A TrialNet Diabetes Onset Adjudication Committee will review all relevant information for each subject who does not meet the criteria stated in section 8.1 but has been otherwise diagnosed as having developed diabetes The Committee will determine whether the diagnosis of diabetes in each of these subjects is sufficiently sound so as to include that subject among the cases who have reached the primary outcome in the statistical analysis. The Committee will be masked to treatment assignment as it reviews each case masked to treatment assignment.

10.3.5. Clinical Site Monitoring

In order to conduct this study with established research principles, site visits will be conducted during the study to evaluate study conduct. All sites will be monitored by the Coordinating Center and appropriate TrialNet committees for patient enrollment, compliance with protocol procedures, completeness and accuracy of data entered on the case report forms (CRFs), and the occurrence and reporting of adverse events (AEs) and serious adverse events (SAEs).

10.4. Medical Monitor and Data Safety and Monitoring Board (DSMB)

All adverse events will be recorded on the adverse event forms, which will be sent to the local IRBs, per their reporting requirements, and to the Coordinating Center.

An independent physician will be designated to serve as the medical monitor for this study who will maintain regular contact with the study and the study chair. (S)he will review all adverse event reports, masked to treatment assignment, and will file event reports with regulatory authorities as appropriate.

The DSMB will meet approximately every 3 months and as needed to review indicators of safety. In addition, they will meet every 6 months to review the interim effectiveness and potential toxicity of the study treatments based on interim analyses of indicators of effectiveness and safety prepared by the Coordinating Center separately by treatment group. The DSMB will independently evaluate whether there are grounds to modify or discontinue the study.

10.5. Sample and Data Storage

Samples to be stored for research purposes will be located at the NIDDK Repository and at TrialNet Sites. While TrialNet is active, the use of the samples will be restricted to TrialNet researchers unless researchers from outside of TrialNet obtain approval from the TrialNet Steering Committee and the NIDDK to utilize the samples. Samples that are obtained for pharmacokinetics and measurement of anti-teplizumab antibodies may be made available to MacroGenics for analysis. All samples will be coded with unique study numbers, but TrialNet researchers will be able to identify samples if it is necessary to contact participants for reasons of health or for notification to them about future studies. Approval from the TrialNet Steering Committee and the NIDDK would be required before such linkage could occur. Researchers from outside of TrialNet will not be permitted to identify samples.

Data collected for this study will be sent to the TrialNet Coordinating Center. After the study is completed, the safety study data will be sent to MacroGenics by the TNCC to allow integration of all safety data on teplizumab. De-identified data will be stored at the NIDDK Repository, under the supervision of the NIDDK/NIH, for use by researchers including those outside of TrialNet.

When TrialNet is completed, samples will continue to be stored at the NIDDK Repository Sites. Since the stored data will be fully de-identified upon the completion of TrialNet, it will no longer be possible to identify samples. Thus, whereas a sample can be destroyed upon a participant's request during the existence of the TrialNet, it can no longer be destroyed once TrialNet is completed. However, there will still be the potential to link data derived from the samples with data that had been derived from TrialNet studies. Once TrialNet is completed, researchers will only obtain access to samples through grant proposals approved by the NIDDK. The NIDDK will convene an external panel of experts to review requests for access to samples.

10.6. Preservation of the Integrity of the Study

The scientific integrity of the trial dictates that results be reported on a study-wide basis; thus, an individual Center will not report the data collected from its site alone. All presentations and publications using TrialNet trial data must protect the main objectives of the trial. Data that could be perceived as threatening the study outcome will not be presented prior to release of the primary study outcomes. Approval as to the timing of presentations of data and the meetings at which they might be presented will be granted by the TrialNet Steering Committee. Study results should be discussed with the news media only upon authorization of the Steering Committee, and never before the results are presented. Any written statements about this study that are shared with national media must be approved by TrialNet before release.

10.7. Participant Reimbursement and Compensation

Participants will be compensated for each visit attended in the study.

APPENDIX 1: Natural History to Teplizumab in At-Risk Relatives Study Flow Chart

Natural History Screening	
Procedures	First or second degree relative Initial AutoAntibody draw
Results to move on	**AutoAntibodies (AA)** At least one autoantibody confirmed positive, or two autoantibodies present

⇩

Natural History Risk Assessment	
Procedures	Confirmation of autoantibody status, OGTT, HLA
Results to move on to teplizumab	**AutoAntibodies (AA)**[1] At least two confirmed diabetes related autoantibodies confirmed to be present on two occasions. Confirmation of 2 positive autoantibodies must occur within the previous six months but the confirmation does not have to involve the same 2 autoantibodies. **OGTT**[2] Fasting Plasma Glucose $\geq$ 110 mg/dL and < 126 mg/dl OR 2-hr Plasma Glucose $\geq$140 mg/dL and < 200 mg/dl OR 30, 60, or 90 minute glucose ≥ 200 mg/dl

⇩

Teplizumab eligibility visit	
Procedures	Screening consent is signed. OGTT, laboratory assessments, PPD, History, PE, Volunteer Quiz, Education.
Results to move on	OGTT with abnormal glucose tolerance Does not meet any exclusion criteria[3]

⇩

Teplizumab Baseline and Randomization visit	
Procedures	Intervention consent signed. Baseline laboratory assessments, dosing of teplizumab/placebo

[1]If autoantibodies are not confirmed positive on the second test a tiebreaker draw will be required.
[2]If the OGTT confirms abnormal glucose tolerance, the subject is eligible to proceed with randomization. If the OGTT is consistent with diabetes, the subject is not eligible for enrollment. He/She may be eligible for enrollment in the future if subsequent studies do not confirm the diagnosis of diabetes and the above entry criteria are met. If neither abnormal glucose tolerance or diabetes is confirmed, the subjects may have repeat studies as outlined above to meet the entry criteria.
[3]If subject not eligible or unwilling to participate in teplizumab in at-risk, subject may be followed in TN Natural History Study.

APPENDIX 2 - Schedule of Assessments

Week of Trial															2		6													
Month of Trial																		3	6	12	18	24	30	36	42	48	54	60	66	72[8]
Day of Trial	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	20														
Visit number	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Study drug[1]		X	X	X	X	X	X	X	X	X	X	X	X	X	X															
Chemistries [2]	X	X	X	X	X	X	X	X					X		X		X		X	X		X		X		X		X	X	X
INR[2]	X	X	X	X	X	X	X						X		X															
CBC with diff[2]	X	X	X	X	X	X	X	X		X			X		X	X	X	X	X	X	X	X		X		X		X	X	X
mAb levels[3]		X										X	X	X	X															
Anti-teplizumab response	X																	X												
EBV and CMV viral loads	X															X	X	X		X		X		X		X				
EBV/CMV serology	X																			X		X		X		X				
History/Physical exam[4]	X	X					X					X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Oral Glucose Tolerance [5]	X																	X	X	X	X	X	X	X	X	X	X	X	X	X
HIV, HepB and C serology	X																													
PPD test	X																													
Urine pregnancy test	X	X																X	X	X	X	X	X	X	X	X	X	X	X	X
HbA1c	X																	X	X	X	X	X	X	X	X	X	X	X	X	X
Mechanistic assessments[6]	X	X				X									X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
glycemic status[7]																														
EKG		X																		X										

1= Drug dosing: Day 0: 51 mcg/m2, Day 1: 103 mcg/m2, Day 2: 207 ug/m2, Day 3: 413 ug/m2, Days 4 - 13: 826 ug/m2.
2= These studies must be reviewed prior to drug administration (see protocol re: drug withholding)
3= These PK samples will be done on the first 12 subjects in each of the age strata: > 16, 12-16, and 8-11.
4= Directed/limited physical exam for visits at month 3, 6, 18, 30, 42, 54, 66, and then q 6 months.
5= If OGTT consistent with DM, repeat within 1 month. Glucose, insulin, and C-peptide are collected at each OGTT.
6=Includes samples for RNA, plasma, serum, DNA, measures of B and T cell number and function to understand the effect of therapy on the immune system and infectious disease. The schedule for these assessments may vary as appropriate. At no time will the blood draw volume exceed what is allowable according to the subject's age and body weight (For subjects <18 5ml/kg per visit, 9.5 mg/kg in an 8 week period.)
7= All subjects will have interim phone contact with study personnel for formal inquiry about adverse events, presence or absence of blurred vision, polyuria, polydypsia, unintended weight loss. In addition, random glucose samples will be obtained at 3 month intervals in which there is no OGTT scheduled. Those with symptoms or glucose >200mg/dl will undergo fasting glucose or OGTT evaluation.
Additional samples will be drawn in the case of drug withholding (see protocol: drug withholding 4.5.2)
8= The assessments at month 72 will be repeated every 6 months.

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ANTI-CD3 MAB (TEPLIZUMAB) FOR PREVENTION OF DIABETES IN RELATIVES AT-RISK FOR TYPE 1 DIABETES MELLITUS

(Protocol TN-10)

VERSION

June 25, 2014

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IND # 102, 629

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Supported by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the National Institute of Allergy and Infectious Diseases (NIAID), the National Institute of Child Health and Human Development (NICHD), the National Center for Research Resources (NCRR), the Juvenile Diabetes Research Foundation International (JDRF), and the American Diabetes Association (ADA).

PREFACE

The TrialNet Type 1 Diabetes Protocol TN-10, Anti-CD3 (teplizumab) for Prevention of Diabetes in Relatives at risk for Type 1 Diabetes Mellitus (T1DM), describes the background, design, and organization of the study. The protocol will be maintained by the TrialNet Coordinating Center (TNCC) over the course of the study through new releases of the entire protocol, or issuance of updates either in the form of revisions of complete chapters or pages thereof, or in the form of supplemental protocol memoranda.

Abbreviations

ADA	American Diabetes Association
AE	Adverse event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUC	Area under the curve
CBC	Complete blood count
CFR	Code of Federal Regulations
CMV	Cytomegalovirus
CRF	Case report form
DCCT	Diabetes Control and Complications Trial
DPT-1	Diabetes Prevention Trial of Type I Diabetes
DSMB	Data and Safety Monitoring Board
EBV	Epstein-Barr virus
FDA	US Food and Drug Administration
GAD	Glutamate decarboxylase
GCP	Good clinical practice
HbA_{1C}	Hemoglobin A1C
HIV	Human immunodeficiency virus
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
ITN	Immune Tolerance Network
NCI-CTCAE	National Cancer Institute *Common Terminology Criteria for Adverse Events*
PE	Physical exam
PPD	Purified protein derivative test
SAE	Serious adverse event
T1DM	Type 1 diabetes mellitus
TNCC	TrialNet Coordinating Center

TABLE OF CONTENTS

1. INTRODUCTION

1.1. Study Overview

Title	Anti-CD3 mAb (teplizumab) for prevention of diabetes in relatives at-risk for Type 1 diabetes mellitus
IND Sponsor	MacroGenics, Inc. Under IND 102,629
Study Supported by	National Institute of Diabetes, Digestive and Kidney Diseases
Conducted By	Type 1 Diabetes Trial Network (TrialNet)
Protocol Chair	Dr. Kevan Herold, Yale University
Accrual Objective	The study plans to enroll approximately 71 subjects over 6 years. The study is projected to last between 6-10 years, depending upon rate of enrollment and number of subjects who develop diabetes.
Study Design	The study is a 2-arm, multicenter, randomized, placebo controlled masked clinical trial. All subjects will receive close monitoring for development of T1DM.
Treatment Description	Subjects will receive teplizumab + close monitoring for development of T1DM or placebo + close monitoring for development of T1DM.
Objective	To assess the safety, efficacy, and mode of action of teplizumab for prevention of T1DM.
Primary Outcome	The primary objective is to determine whether intervention with teplizumab will prevent or delay the development of T1DM in high risk autoantibody positive non-diabetic relatives of patients with T1DM.
Secondary Outcome	Secondary outcomes are to include analyses of C-peptide and other measures from the OGTT; safety and tolerability; and mechanistic outcomes.
Major Inclusion Criteria	Autoantibody positive relatives of T1DM proband with abnormal glucose tolerance. Age 1-45 years at the time of enrollment in TN01 and age ≥ 8 at time of randomization in this trial.

1.2. Statement of Purpose

This protocol describes the background, design, and organization of study of the anti-CD3 monoclonal antibody, teplizumab [hOKT3γ1(Ala-Ala)] for prevention of diabetes in relatives at very high risk for T1DM. The protocol was written by Dr. Kevan Herold, Chair of the TrialNet Anti-CD3 Protocol Committee, the TrialNet Chairman’s Office at the University of Miami and the Benaroya Research Institute, and the TNCC. Significant changes that occur to this protocol during the course of the trial require the formal approval of the TrialNet Steering Committee. The study protocol, along with the required informed consent forms, will be approved by each participating institution’s Institutional Review Board (IRB) or Ethics Committee/Research Ethics Board (EC/REB).

2. BACKGROUND AND SIGNIFICANCE

2.1. Type 1 Diabetes (T1DM)

2.1.1. Definition and metabolic characteristics of Type 1 diabetes mellitus

Type 1 diabetes mellitus (T1DM) is an immune-mediated disease in which insulin-producing beta cells are completely or near completely destroyed, resulting in life-long dependence on exogenous insulin. It is a chronic and potentially disabling disease that represents a major public health and clinical concern. The number of patients being diagnosed with type 1 diabetes is increasing each year and is approaching an epidemic level in some countries that track this information (1; 2).

Compared to individuals with the more common form of diabetes, Type 2 diabetes, (where individuals retain some endogenous insulin production which is inadequate to maintain normal glucose and lipid metabolism), the metabolic impairment in T1DM is much more severe and the loss of insulin production more complete. Continuous exogenous insulin therapy is needed to prevent ketoacidosis and allow assimilation of food and to maintain life. Most likely as a consequence of the absolute deficiency of insulin, glucose counterregulation (i.e. the hormonal response to insulin induced hypoglycemia) is impaired, and therefore, hypoglycemia is a frequent complication of the disease. The occurrence of hypoglycemia limits the ability to achieve near normal glucose control. The Diabetes Control and Complications study (DCCT) showed that the long term complications could be reduced with near normal control of glucose levels but at the cost of an increased frequency of severe hypoglycemia (3). While there have been significant improvements in insulin delivery systems, such as continuous subcutaneous insulin infusions with insulin pumps, normal glucose control, particularly in children, is rarely achieved. Therefore, individuals with Type 1 diabetes remain at risk for secondary end-organ complications including visual impairment and blindness, renal failure, vascular disease and limb amputation, peripheral neuropathy, stroke, acute risk for severe hypoglycemia, and others. Moreover, at the time of diagnosis, many individuals, and children in particular, suffer significant morbidity frequently requiring ICU admission. As described below, virtually all the individuals identified for enrollment into this prevention trial will develop diabetes. Clearly, prevention of the onset of the disease itself would represent a significant advancement.

2.1.2. Natural History of Type 1 Diabetes

Much is known about the natural history of the type 1 diabetes disease process (4). Although all people are susceptible, relatives of individuals with T1DM are at much greater risk for development of the disease. In the general population, approximately 0.3 % of individuals will develop T1DM. In contrast, those with a relative with T1DM have a 5% incidence of disease – a 15 fold increase (5). Further risk stratification among family members depends upon genetic, immune and metabolic data (6).

Beta cell destruction generally begins in genetically susceptible individuals years before clinical onset(7). The autoimmune process that causes beta cell destruction is clinically silent and can only be identified by the detection of autoantibodies such as Islet Cell Antibodies (ICA), anti-glutamic acid decarboxylase (GAD)65ab, anti-ICA512ab, anti-insulin autoantibodies (mIAA) (5), and the recently described antibodies to a zinc transporter (ZnT8) (8). Continued immune mediated beta cell destruction occurs until physiologic insulin demand cannot be met by the remaining beta cells, resulting in hyperglycemia and clinical diagnosis of T1DM (9)(10).

Based on data from the Diabetes Prevention Trial, type 1 diabetes (DPT-1), the risk for developing diabetes in relatives without the disease can be defined by the presence of autoantibodies and the degree of metabolic impairment (11-13). The DPT-1 study was one of the first large-scale prevention trials of T1DM. The aim of this trial, which tested >100,000 relatives of individuals with T1DM, was to study whether either low dose parenteral insulin or oral insulin administration would prevent the development of T1DM. The results of the DPT-1 study showed that neither parenteral nor oral insulin prevented the development of T1DM, (although a secondary analysis of the data suggested some effect of oral insulin in delaying the onset of diabetes in a subgroup of subjects defined by high anti-insulin antibodies and normal glucose tolerance)(13).

Autoantibody positive subjects enrolled in the DPT-1 study who had impaired or indeterminate glucose tolerance (any glucose level after ingestion of oral glucose of > 200 mg/dL and/or a glucose level 2 hours after ingestion of oral glucose of 140-200 mg/dL and/or fasting glucose between 110 – 126 mg/dL during a standard oral glucose tolerance test were at very high risk (78% over 5 years) of developing T1DM over a 5 – 6 year follow up. The risk was particularly high for individuals under the age of 18 (Figure 1).

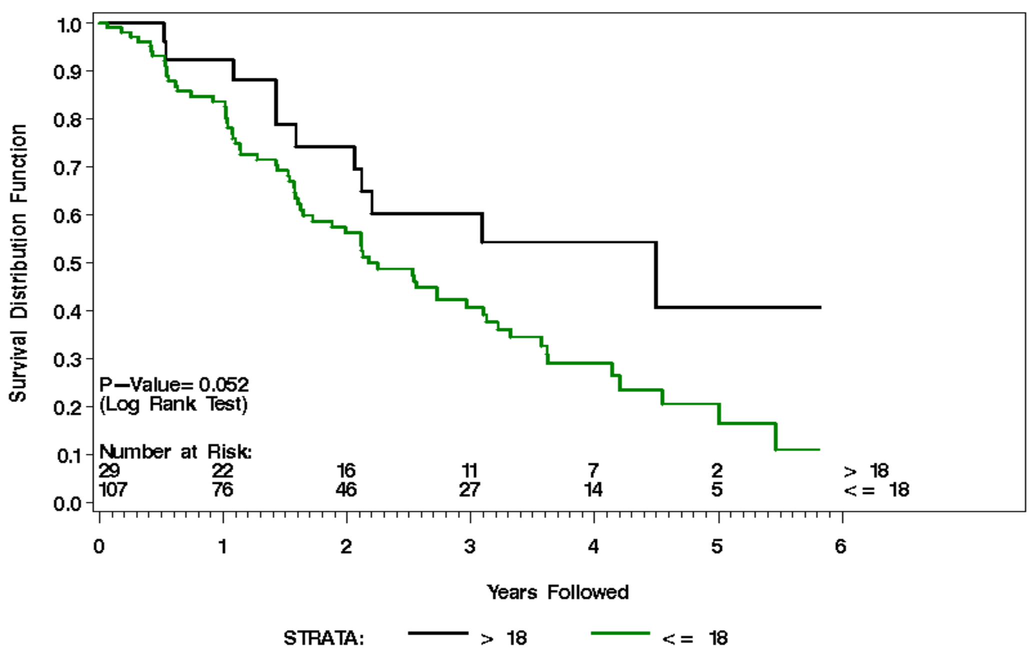


Figure 1: Risk of diabetes among individuals recruited for the DPT-1 study with abnormal glucose tolerance, stratified by age≤ 18 or ≥ 18 yrs.

Similar results confirming the very high risk of those with abnormal glucose were found in the ENDIT (European Nicotinamide Diabetes Intervention Trial) study in which Nicotinamide failed to prevent the onset of diabetes in relatives at risk for the disease (14).

It is important to note that the diagnosis of diabetes is based on a glucose threshold that is associated with risk of secondary endorgan complications of the disease rather than the pathologic process that leads to hyperglycemia. Detailed analyses of metabolic function in individuals who do and do not progress to diabetes in the DPT-1 study have been published (10; 15; 16). These studies have identified the progressive loss of stimulated C-peptide responses to a mixed meal over time in high risk individuals. The differences between the responses in the prediabetic period and after the diagnosis of diabetes are modest but statistically significant. These studies describe a progressive predictable loss of beta cell function rather than a precipitous change. In addition, they also suggest that once metabolic impairment has occurred, the risk is extremely high. These combined immunologic and metabolic studies suggest to us that those individuals differ from those in whom the diagnosis of T1DM has been made only in the time of progression.

There have been no therapies tested to date which are aimed only at those at very high risk for development of T1DM (~80% as described above). The previous DPT-1 and ENDIT studies enrolled subjects with a broad range of risk – the overall 5-year risk in the target population was between 50-60%. However, in newly diagnosed subjects, there is reason to believe that individuals with more beta cell function may show a better response to interventions. Earlier studies with Cyclosporine A suggested that response to immune therapies is greatest in those with higher levels of insulin secretion at the time of diagnosis of T1DM (17). In a recently published study of another non-FcR binding anti-CD3 mAb, Keymeulen et al found that clinical responses to drug were greatest in those in the upper half of C-peptide responses at the time of study entry (18).

Therefore, the rationale for this study is that individuals with immunologic markers of T1DM and abnormal glucose tolerance are at very high risk for progression to overt disease. They have a condition that differs from overt diabetes only in the duration of the autoimmune process that results in beta cell destruction. Intervention at the “prediabetic” stage is likely to be more effective than intervention in those in whom frank hyperglycemia has developed and beta cell function has deteriorated further because insulin production is greater before compared to after the diagnosis.

2.2. Development of Teplizumab

The Fc-engineered teplizumab [hOKT3γ1 (Ala-Ala)] was developed as an approach to mitigate the adverse effects of OKT®3 resulting from Fc/FcR engagement (19). OKT®3 produces profound, transient T-cell depletion in vivo. It also activates T cells, is strongly mitogenic, and its use in vivo is associated with severe cytokine-release syndrome (incidence >90%). The cytokine-release syndrome induced by OKT®3 is characterized by fever, chills, nausea, vomiting and other symptoms, and usually requires corticosteroid therapy to suppress. OKT®3 also is associated with a small incidence of EBV lymphomas (~1%-2%). T-cell activation is strongly facilitated by the interaction of Fc component of OKT®3 with Fc receptors on lymphocytes (Fc/FcR engagement).

Teplizumab is a 150-KD humanized mAb that binds the CD3-e epitope of the T cell receptor (TCR) complex with affinity equal to OKT®3, but it differs from OKT®3 in two properties:

1. The humanization process has resulted in the generation of a mAb that used less than 10% of the original murine amino acids in the antibody construction. The clinical consequence of this property is reduced immunogenicity or formation of anti-idiotypic antibodies.

2. Two amino acids have been changed (leucine$_{234}$ to an alanine and leucine $_{235}$ to an alanine) in the Fc portion of the immunoglobulin that disrupt Fc receptor and complement component C1q

binding. These two amino acid changes were aimed at eliminating the majority of cytokine-mediated toxicity observed during infusions of OKT®3.

The modified Fc component of teplizumab minimizes the activating capacity of the antibody compared with unmodified murine OKT®3. Although the primary mechanism of action of the antibody involves binding the CD3 antigen target on T cells, subsequent mechanisms involved in the therapeutic effects are incompletely understood. These mechanisms of action appear to involve weak agonistic activity on T cells as well as the generation of regulatory cytokines and regulatory T cells leading to the development of tolerance (20; 21).

2.3. Clinical Studies

As of March 2009, over 500 subjects have been treated with teplizumab including over 450 individuals with T1DM. Other subjects who have received teplizumab include subjects undergoing renal allograft rejection, individuals receiving islet transplantation, and patients with psoriatic arthritis.

Two clinical trials testing safety and efficacy have been completed using teplizumab in participants with recent onset T1DM; Study 1 (a phase I/II trial); Study 2 (protocol ITN007AI [NDB01]), as well as one PK/safety study (protocol ITN017AI). In addition, four clinical studies to preserve beta cell function in those with T1DM are underway. These studies are described below. Further information about these studies and other clinical experience with teplizumab are in the Investigator's Brochure.

2.3.1. Study 1: A Phase I/II Trial

Study 1 was a randomized, controlled, phase I/II, three-center trial that enrolled a total of 43 participants and tested two dosing regimens with hOKT3γ1(Ala-Ala)(23; 30). The clinical efficacy outcome tested was change in C-peptide response to MMTT in treated as compared to control groups.

The results of these studies suggest that treatment with the anti-CD3 mAb hOKT3γ1 (Ala-Ala) reduces the loss of insulin production over the first year in individuals with T1DM (Table 1)(23; 30).

***Table 1**. Changes in C-peptide response to an MMTT among participants in study 1*

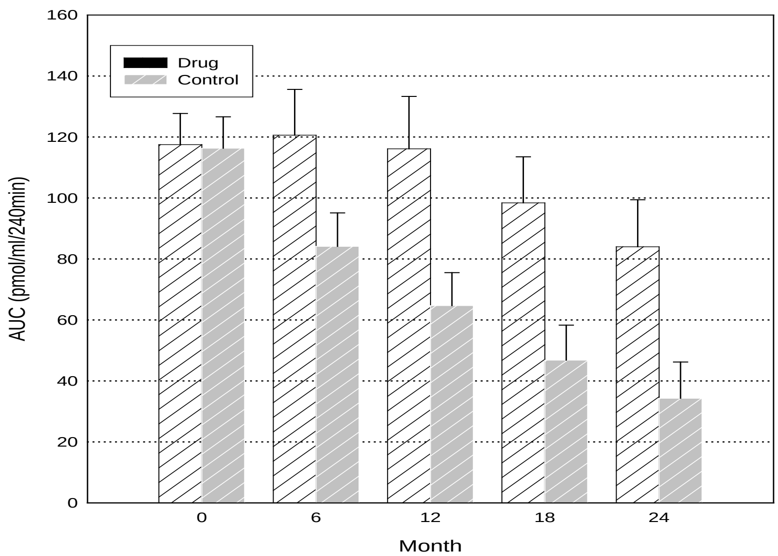
Patient Group	Change at 6 months			Change at 1 year		
	↑	No change	↓	↑	No change	↓
Drug treated	9	6	6	7	8	6
Control*	2	2	15	1	3	15

p<0.01; * Two control participants withdrew from the study.

At 1 year, the mean area under the curve (AUC) of the C-peptide response was 97±9.6% of the response at baseline in the drug-treated group (vs. 53±7.6% in the control group, p=0.001).

Follow-up was extended for 2 years after study entry. There continued to be a significant drug treatment effect at 2 years (p=0.002), although the meal stimulated C-peptide responses were falling in both the drug treated and control groups. The differences between the two groups were 44% at both year 1 and year 2 (31).

***Figure 2**. C-peptide response to an MMTT over 2-year period in study 1. There was a significant effect of drug treatment on the C-peptide responses over the 2 year period (p=0.002). (The data represent mean ± SEM of the study groups.)*



In the patients with diabetes, the drug treatment was associated with improvement in the hemoglobin A_{1C} (HbA_{1C}) levels (p=0.004) and reduced use of insulin (p=0.001) over the 2-year period.

2.3.2. Study 2: A Phase II Multiple-Dose Trial, NCT00806572

Protocol ITN007AI [NDB01]) started in June 2002 to assess the ability of the regimen to prolong the duration of the clinical response and to increase the number of responders (31). In study 2, the drug was to be administered soon after clinical diagnosis and at 6 and 12 months after diagnosis. Between June and August 2002, 10 patients were enrolled in study 2 (6 were randomized to the drug treatment group and 4 to the control group) and those assigned to the drug treatment group received one course of hOKT3γ1 (Ala-Ala) treatment. Further enrollment and hOKT3γ1 (Ala-Ala) treatments were stopped based on adverse event findings. Further investigation into the potential differences of the drug products and their preparations indicated that the absolute amount of drug that was administered to the six experimental patients in study 2 was greater than had been used in study 1.

2.3.3. ITN 017: A Phase I dosing study

Following discontinuation of Protocol ITN007, protocol ITN017 was conducted to determine a safe dose to be used for studies of teplizumab in subjects with Type 1 diabetes. Six subjects were enrolled in this open label trial. There were no serious adverse events in the first dosing cohort of 4 subjects. The study was discontinued when the first subject in the higher dosing cohort developed hyperbilirubinemia. The current dosing regimen is based on the findings from ITN017.

2.3.4. "ABATE Trial" ITN027 NCT00129259

This Phase II randomized open label study will test whether two courses of treatment (each for 14 days) within 8 weeks of diagnosis and at 1 year will improve C-peptide responses 2 years after diagnosis. There have been 6 serious adverse events in this trial. Three of these were judged to be study drug related. Two of the 3 were lymphopenia that was not unexpected, and one was cytokine release syndrome. This study showed reduced decline in C-peptide in the drug-treated vs control group (p=0.001, manuscript in preparation).

2.3.5. "Delay Trial" NCT00378508

This randomized, double blind, placebo controlled trial will test the ability of a single 14 day course of treatment with teplizumab to prevent the loss of C-peptide 1 year after study enrollment. The "Delay Trial" offers optional retreatment for all subjects who retain detectable levels of insulin production, 1 year after study entry. The inclusion criteria for the Delay Trial differs from other studies in its enrollment of individuals with Type 1 diabetes of duration of 4 – 12 months. There have been two serious adverse events in this trial, neither of which was judged to be study drug related. The analysis of the primary endpoint (difference in C-peptide responses to a mixed meal test at 12 mos adjusted for baseline C-peptide response and duration stratum) showed significantly greater responses in the teplizumab treated group (p=0.03, manuscript submitted). However, because of an imbalance in HgbA1c levels at baseline, an additional analysis was performed correcting for the baseline HgbA1c level, C-peptide AUC, and duration stratum, and difference in the primary endpoint was 17.7% (p=0.09). Nonetheless, the drug treated group lost significantly less of the baseline C-peptide AUC level (20.6%) compared to placebo treated subjects (37%)(p=0.04) after correction for baseline C-peptide AUC, baseline HgbA1c, and duration stratum, and a greater proportion of subjects lost detectable C-peptide in the placebo group (p=0.02). In a post-hoc analysis subjects with HgbA1c levels < 6.5% and younger (< age 15 yrs) subjects showed significant responses to teplizumab after correction for duration strata and baseline C-peptide and baseline HgbA1c levels (as a covariate in the analysis of age strata).

2.3.6. "Protégé Trial" NCT00385697

This is a Phase II/III multicenter study to compare efficacy, safety, and tolerability of 3 dose levels of teplizumab relative to placebo, in subjects within 12 weeks of T1DM diagnosis. Segment 1 was an open label study of 30 subjects which has completed enrollment.

The results from the analysis of the primary endpoint were announced in October 2010 and published in 2011. The primary endpoint (a comparison of the proportion of subjects with hemoglobin A1C<6.5% who were using < 0.5U/kg/d of exogenous) was not met. However, a posthoc analysis of the effects of full dose of drug treatment showed that the C-peptide levels in the teplizumab treatment group were significantly greater than in placebo treated control subjects 12 months after enrollment (Sherry et al, 2011).

2.3.7. "Protégé Encore Trial" NCT00385697

This is a Phase II/III multicenter study to compare efficacy, safety, and tolerability of 3 dose levels of teplizumab relative to placebo, in subjects within 12 weeks of T1DM diagnosis. Enrollment into this double-masked, placebo controlled, four arm trial was ended in October 2010, prior to completing enrollment. A follow up study of subjects is underway.

2.4 Evaluations of Safety Experience with Teplizumab and the Basis for the Proposed Clinical Protocol

Safety reviews of the use of teplizumab in patients with type 1 diabetes were carried out in 2004, 2006, and continue on an ongoing basis by Data Safety and Monitoring Boards. These evaluations identified the occurrence of mild cytokine release, during the first 6 days of drug treatment, in about 10% of subjects. The 2006 safety evaluation noted that study drug was discontinued in 6/60 individuals due to adverse events characterized by laboratory abnormalities of consumptive coagulopathy and were thought to be due to the release of cytokines that occurred with the initial doses of drug. Specifically, the signs that identified this complex included any of the following: a) fever (of grade 3), b) an increase in the PT and/or PTT and an increase in the level of D-dimers, c) an increase in the total bilirubin level, d) hypotension, and e) an increase in liver enzymes. In each case, the abnormal findings began within the first 6 days of drug administration, and frequently with the first dose of drug. In all 6 cases, drug administration was stopped prior to administration to a complete course of protocol-defined treatment. The laboratory abnormalities were self-limited, did not progress, and completely resolved without sequelae.

Based on these analyses, additional laboratory studies to evaluate the presence of cytokine release were recommended, and more stringent criteria for withholding drug were developed. With the modified criteria, all subjects who discontinued drug administration because of adverse events were identified by the second dose of drug. These new evaluations are incorporated into this and other ongoing protocols. More information is available in the Investigator's Brochure.

2.5. Use of Teplizumab in Children

The majority of new cases of T1DM occur in children under the age of 18. Moreover, given that the duration of diabetes is a significant risk factor for the development of diabetes complications and the clearly recognized difficulty in attaining excellent metabolic control of diabetes during adolescence (3; 32), it is critical that children are included in studies of the prevention of type 1 diabetes. The experience gained from DPT-1 and other studies demonstrates that children are more likely to have risk factors associated with rapid progression of the disease, suggesting a difference in the natural history of the disease from that in adults, and again demonstrating the need to include children in prevention studies.

600 subjects have participated in clinical trials of teplizumab: 100 in open-label studies (all treated with teplizumab) and approximately 500 in randomized, blinded studies (approximately 80% treated with teplizumab). See **Table 2** shown below which displays enrollment by age group.

***Table 2**: Age Distribution of Subjects by Study in Previous and Ongoing T1DM Studies (Data Available as of July 2009)*

	Open-label (Treated with Teplizumab)						Blinded		Open-Label & Blinded
Age Range (years)	**Herold Study 1**	**ITN 007 (NDB01)**	**ITN017**	**ITN027 (Abate)**	**Protégé Segment 1**	**Total**	**Delay**	**Protégé Segment 2**	**Total**
8-11	9	2	2	24	11	**48**	7	73	**128**
12-17	8	1	4	23	16	**52**	9	189	**250**
≥18	5	3	0	4	11	**23**	2	230	**255**
Total	**22**	**6**	**6**	**51**	**38**	**123**	**18**	**492**	**633**

Notes: Protégé is Protocol CP-MGA031-01. The Herold studies are investigator-initiated studies. For further information regarding these studies, please refer to the Investigator's Brochure

Dr. Herold and colleagues reviewed the adverse event experience of the completed trials and concluded that although the number of subjects was small, there was no apparent relationship of age to the number or severity of adverse events.

In addition to the possibility of delaying or preventing the onset of T1DM, which is unproven, there are other prospects for direct benefit to children by their participation in the study. These include the recognized benefits of being in a clinical study, and of close monitoring for development of diabetes which significantly prevents morbidity associated with onset of the disease in the community including ICU admissions. The intervention has the prospect of direct benefit to the individual subject and in addition, is likely to yield general knowledge about T1DM which is of importance for the understanding and amelioration of T1DM in children.

The study procedures, while greater than minimal risk, offers the possibility of benefit due to the close monitoring for all participants, including children. Assent of children along with consent of the parents will be obtained prior to any study procedures. This research proposal in children is therefore consistent with United States Department of Health and Human Services, Protection of Human Subjects, 45CFR46.405 (research involving greater than minimal risk but presenting the prospect of direct benefit to individual subjects) and with 21CFR50.52 (Clinical investigations involving greater than minimal risk but presenting the prospect of direct benefit to individual subjects).

2.6. Additional information

Please refer to the Teplizumab Investigator's Brochure for further non-clinical and clinical information on the antibody.

3. STUDY DESIGN

3.1. Overview

This is a multicenter, double masked, randomized, placebo- controlled study to determine whether treatment of subjects at high risk for diabetes with teplizumab results in delay or prevention of clinical T1DM.

3.2. Objectives

3.2.1. Primary Objective

The primary objective of the study is to determine whether treatment of at-risk subjects with teplizumab results in a delay or prevention of T1DM.

3.2.2. Secondary Objectives

- to determine whether treatment with teplizumab is superior to placebo with respect to C-peptide responses to oral glucose, as obtained from timed collections during longitudinal tests
- to compare the safety and tolerability of teplizumab to placebo
- to assess the effects of treatment on mechanistic outcomes

3.3. Summary of Inclusion/Exclusion Criteria

Participants must meet all entry criteria for the protocol as outlined below.

3.3.1. Inclusion Criteria:

Study subjects must be or have:

1. Participant in TrialNet Natural History Study (TN01) and thus a relative of a proband** with T1DM.
2. Between the ages of 1-45 years at the time of enrollment in TN01 and age ≥ 8

   at time of randomization in this trial
3. Subject (or parent or legal guardian if the subject is a minor) is willing to provide Informed Consent.
4. Individuals <18 years of age at time of randomization must have had a TrialNet conducted OGTT demonstrating abnormal glucose tolerance* within 7 weeks (52 days) of the baseline visit (visit 0).
5. Individuals ≥18 years of age at time of randomization must have had two consecutive TrialNet conducted OGTT's demonstrating abnormal glucose tolerance, the most recent of which must have been within 7 weeks (52 days) of the baseline visit (visit 0).
6. The participant must be positive for two or more diabetes-related autoantibodies on two occasions.

The second occasion must occur within the six months prior to study drug administration, but does not need to involve the same two autoantibodies as were found on the first occasion. The autoantibodies that are to be confirmed are anti-GAD65, anti-ICA512, anti-insulin (MIAA), ZnT8 and/or ICA.

7. Weigh at least 26 kg at randomization.

8. If participant is female with reproductive potential, she must have a negative pregnancy test on Day 0 and be willing to avoid pregnancy for at least one year from randomization.

9. If participant is male, he must be willing to avoid pregnancy in any partners for at least one year from randomization.

10. Willing and medically acceptable to postpone live vaccine immunizations for one year after treatment.

11. Willing to forego other forms of experimental treatment during the study.

* a. Fasting plasma glucose ≥ 110mg/dL, and <126mgdL

or

b. 2 hour plasma glucose ≥140mg/dL, and <200mg/dL

or

c. 30, 60, or 90 minute value on OGTT ≥200mg/dL

Fasting glucose levels of 110-125 mg/dL qualify subjects as having abnormal glucose tolerance in this protocol as it reflects the criteria used for entry into the DPT-1 (33) study and the DPT-1 data was used for the calculation of diabetes risk for this trial. Using data for individuals with Type 2 diabetes, the ADA uses a different glucose range to define impaired fasting glucose(34).

** A proband is an individual diagnosed with diabetes before age 40 and started on insulin therapy within one year of diagnosis, or if subjects with probands considered to have type 1 diabetes by their physician who do not meet this definition, the TrialNet Eligibility Committee for the TrialNet Natural History/Pathway to Prevention Study (TN01) must have approved enrollment in TN01.

3.3.2. Exclusion Criteria:

Study subjects cannot be randomized if they have:

1. If ≥18yo: Diabetes, or have a screening OGTT with:
    a. Fasting plasma glucose ≥126 mg/dL, or
    b. 2 hour plasma glucose ≥ 200mg/dL
2. If <18yo: Diabetes, or have a screening random glucose ≥200mg/dL
3. Lymphopenia (< 1000 lymphocytes/µL).
4. Neutropenia (< 1500 PMN/µL).
5. Thrombocytopenia (< 150,000 platelets/µL).
6. Anemia (Hgb < 10 grams/deciliter [g/dL]).
7. AST or ALT >1.5 x ULN.
8. Total bilirubin >1.5 x upper limit of normal (ULN) with the exception of subjects with the diagnosis of Gilbert's syndrome who may be eligible provided they have no other causes leading to hyperbilirubinemia.
9. INR > 0.1 above the upper limit of normal at the participating center's laboratory.
10. Chronic active infection other than localized skin infections.
11. A positive PPD test.
12. Vaccination with a live virus within 8 weeks of randomization
13. Vaccination with a killed virus within 4 weeks of randomization.
14. A history of infectious mononucleosis within the 3 months prior to enrollment.
15. Laboratory or clinical evidence of acute infection with EBV or CMV.
16. Serological evidence of current or past HIV, Hepatitis B or Hepatitis C infection.
17. Be currently pregnant or lactating, or anticipate getting pregnant.
18. Chronic use of steroids or other immunosuppressive agents.
19. A history of asthma or atopic disease requiring chronic treatment.
20. Untreated hypothyroidism or active Graves' disease at randomization.
21. Current use of non-insulin pharmaceuticals that affect glycemic control.
22. Prior OKT®3 or other anti-CD3 treatment.
23. Administration of a monoclonal antibody within the year before randomization.
24. Participation in any type of therapeutic drug or vaccine clinical trial within the 12 weeks before randomization.
25. Any condition that, in the opinion of the investigator, would interfere with the study conduct or the safety of the subject.

3.4. Enrollment

Potential study subjects will be identified through the ongoing TrialNet Natural History/Pathway to Prevention study. In this study, first and second degree relatives of patients with T1DM are screened for biochemical autoantibodies and ICA. Those individuals who test positive are then further staged with the performance of an OGTT. The results of this OGTT performed in a TrialNet study will be used to determine eligibility for this protocol.

The TrialNet Natural History/Pathway to Prevention study screens participants at over 200 clinical sites. The infusion of teplizumab or placebo will occur at a limited number of designated TrialNet infusion sites, whereas the initial screening visit and follow-up testing, as described in the Schedule of Assessments, may occur at other (non-infusion)TrialNet sites.

3.5. Double-Masking and Description of Treatment Groups

The intervention will be conducted only amongst those who consent to participate. Subjects will be randomized to receive either teplizumab or placebo. All subjects will undergo close monitoring for the development of diabetes. Subjects and clinical investigators will be masked as to treatment assignment. The intervention protocol will be conducted at approved TrialNet clinical sites with appropriate facilities. All blood and serum samples for the primary and secondary outcome determinations will be sent to the TrialNet Core Laboratories for analysis. Clinical laboratory studies that will be used for determining eligibility for study drug infusion will be performed at the local sites.

Participants will be randomly assigned in a 1:1 ratio (within the two strata defined by age at enrollment: <18 and 18 or older) to the following 2 groups:

- to receive teplizumab (14 day IV infusion) followed by close monitoring for T1DM development
- to receive placebo (14 day IV infusion) followed by close monitoring for T1DM development

3.6. Treatment Assignment

After participants provide informed consent, complete the screening visit(s), and meets all of the inclusion criteria and none of the exclusion criteria, participants will be randomized to receive either teplizumab or placebo.

Participants will be randomized in equal allocations to each group. The randomization method will be stratified by TrialNet study site and whether the participant is less than 18 years of age or 18 years and older. This approach ensures that study site will not be a potential confounder. The TNCC will generate the randomization numbers and tables.

3.6.1. Procedures for Unmasking

Emergency unmasking will occur upon notification of the TrialNet Central Pharmacy and TNCC via the 24 hour emergency number and approval by TrialNet Chair, NIDDK TrialNet program officer, or TrialNet Medical Monitor. Non-emergent unmasking will occur upon notification of the TNCC and approval by TrialNet Chair or NIDDK TrialNet program officer. If unmasking is approved, the study sponsor and appropriate TrialNet committees (e.g. Safety Monitoring) will be notified of the event as soon as reasonably possible; however, they will not be unmasked.

3.7. Study Assessments

During the course of the study, participants will frequently undergo assessments of their glucose tolerance status, insulin production, immunologic status, and overall health and well-being (see Schedule of Assessments).

Samples will be drawn for storage in the National Institute for Diabetes and Digestive and Kidney Disease (NIDDK) Repository and at TrialNet Laboratory Sites for future analysis.

3.8. Quality Assurance

During the study, duplicate collections of blood samples for assays will be obtained in a small sample of subjects for the purpose of external quality surveillance of the performance of the TrialNet Central Laboratories.

3.9. Study Timeline

3.9.1. Study Duration

The study is designed to provide 80% statistical power to detect a 60% risk reduction with a one-sided test at the 0.025 significance level. This risk reduction is expected to result in a delay in the median time to onset of diabetes of 7.52, 8.93, and 10.6 yrs. for the age cohorts < 18 and ≥18 within strata 1, 2, and 3, respectively. To attain these design parameters will require the observation of 40 participants that are diagnosed on-study with T1DM. Consequently, the total sample size and study duration can only be approximated. The study plans to enroll approximately 71 subjects over approximately 6 years, and is projected to last between 6 and 10 years. As the study progresses, projections of the study end will be computed and updated based on the rate of enrollment, the observed hazard rate and the rate of loss-to-follow-up.

3.9.2. Follow-up Studies

Although subjects who develop diabetes will have reached the study endpoint, these individuals will be offered annual follow up for a minimum of two years Those individuals who have not developed diabetes by study end will continued to be followed as part of the TrialNet Natural History/Pathway to Prevention study protocol.

Individuals who develop T1DM may be eligible for interventional studies sponsored by TrialNet or other organizations under separate INDs. In the event that a subject wishes to participate in another investigational study that has, as an exclusion, treatment with experimental or immune modulatory drugs, the subject may request and be told of their treatment group assignment for the anti-CD3 prevention study. Every attempt will be made to minimize potential bias that this may introduce. The TNCC will make treatment assignment information available to the site investigator of the new study after the subject is determined to be willing to participate and not otherwise excluded from the new study. Other study group members will not be informed of the treatment assignment information. Mitigation of bias issues must be balanced against safety and interests of participants.

4. PATIENT MANAGEMENT

4.1. Screening Visit and Eligibility Assessment

This study will draw participants from the TrialNet Natural History/Pathway to Prevention Study.

The initial testing for autoantibodies, HLA type, and Oral Glucose Tolerance Test (OGTT) will be done as part of Natural History/Pathway to Prevention. Those individuals with two confirmed diabetes-related autoantibodies and abnormal glucose tolerance on the OGTT, will then be eligible for additional tests and possible enrollment into this study.

Appendices 1 and 2 summarize the flow of subjects from the Natural History/Pathway to Prevention Study into this study.

4.2. Anti-CD3 mAb Trial for At-risk Subjects Initial Visit

Prior to the initial visit, this study will be described to the potential participant. The participant/parent/guardian will be asked to sign an informed consent document describing the purpose, risks, and benefits of screening for the trial. A participant's signature indicates that he/she understands the potential risks and benefits of study participation. Subjects less than age 18, qualify for the study with a single OGTT with abnormal glucose tolerance. Those age 18 or older, require two consecutive OGTTs with abnormal glucose tolerance. Qualifying OGTTs may occur under the auspice of another TrialNet study or as a part of this visit if needed. During these visits, other clinical tests will also be performed to determine eligibility.

Any participant either not eligible or not willing to be randomized into this study is eligible for continued follow-up as part of the TrialNet Natural History/Pathway to Prevention Study.

4.3. Randomization and Baseline Visits

Review will be made to be certain the subject meets study eligibility criteria. Prior to randomization, the intervention and follow-up phases of the study will be described to the participant. The participant/parent/guardian will be asked to sign an informed consent document indicating that he/she understands the study as well as the potential risks and benefits of study participation.

Participants will be randomized to either the treatment arm or the control arm. The randomization and the baseline visit must occur within 7 weeks of the qualifying OGTT in order to ensure that participants have abnormal glucose tolerance at time of randomization and study drug administration. Note, subjects who are febrile at the time of baseline visit, may have the visit postponed up to five days outside the 7 week window if needed because of intercurrent illness. At or prior to the baseline visit (visit 0), subjects < age 18 will undergo an OGTT.

4.4. Close Monitoring

During the study period, all participants will receive close monitoring for development of diabetes. OGTT tests will be performed at 3 and 6 months and every 6 months thereafter. In addition, at three month intervals (where there is no OGTT scheduled), a random (non-fasting) glucose level will be measured. At each visit and via routine contact in between visits, participants will be asked directed questions about the presence or absence of symptoms associated with diabetes such as blurry vision, unintended weight loss of more than 3 kg, polyuria, and polydypsia. If subjects respond affirmatively to any of these questions or if any of the post-prandial glucose values are greater than 200 mg/dL, further evaluation, including fasting glucose or an OGTT, will be performed. Individuals in both of the study arms will have laboratory and mechanistic studies performed as detailed in the Schedule of Assessments.

4.5. Administration of Teplizumab

4.5.1. Drug Administration

Teplizumab or saline will be administered via IV infusion over 14 days. The 14 day course must commence within 7 weeks of the qualifying OGTT, except in the case of interim illness as noted above (Section 4.3).

Subjects will be randomized to 1 of 2 treatment arms and will receive a 14-day course of teplizumab and/or placebo. The treatment course will be administered on Study Days 0–13. The 2 treatment arms are described below.

- **Arm #1:** Subjects will receive a 14-day course of teplizumab consisting of daily IV doses of 51 micrograms/meter squared (µg/m^2), 103 µg/m^2, 207 µg/m^2, and 413 µg/m^2 on Study Days 0–3, respectively, and one dose of 826 µg/m^2 on each of Study Days 4–13. The total dose for a 14-day course is approximately 9034 µg/m^2. For subjects weighing 70 kg and having a body surface area (BSA) of 1.92 m^2, this dosing schedule delivers ~18 milligrams (mg) of teplizumab.
- **Arm #2:** Subjects will receive a 14-day course of IV placebo only.

The subject's BSA will be calculated by the pharmacist using the Mosteller formula. The BSA will be calculated on Study Day 0, and will be based on the subject's height and weight on that day. This calculation will be used for dosing over the entire 14 days. For dosing purposes the participant's BSA should be rounded up to the nearest tenth place regardless of the number in the hundredth position. For example, if the BSA is 1.61, it will be rounded up to 1.7 and if the BSA is 1.67, it will also be rounded up to 1.7. The dose should be rounded to the nearest whole number. For example if the participant's BSA is 1.722, on Day 0 the participant would receive 92µg.

The Mosteller formula
BSA (m²) = ([Height(cm) x Weight(kg)]/ 3600)^{½}

Teplizumab or saline is administered via an IV infusion over a minimum of 30 minutes in the research or hospital setting. This infusion should be given at the same time each day +/- 4 hours. Vital signs will be monitored for 2 hours after each infusion. Subjects are permitted to leave the research or hospital setting each day upon completion of the 2 hours of post-infusion monitoring if they remain within approximately one hour (driving time) of the treatment center.

The formulation of teplizumab will consist of:

- 10 mM sodium phosphate
- 150 mM sodium chloride
- 0.05 mg/mL Tween 80
- pH 6.1

Final drug product will be provided at a concentration of 1 mg/mL for a total of 2 mg of recoverable drug product per vial.

The vials should be stored upright at 2°–8° C and must not be frozen. Because there is no preservative and drug loss occurs over time, administration of study drug should begin as soon as possible and no later than 2 hours after preparation. The infusion must be complete within 6 hours of preparation. The drug may be prepared into a bag for infusion or into a syringe for delivery by infusion pump. Intravenous drug delivery devices, including IV bags and tubing, must be composed of PVC.

Laboratory studies that will be obtained prior to each dose are described in the Schedule of Assessments. The results of chemistries including liver function tests, WBC, Hgb, Hct, platelets, and INRs must be reviewed each day they are drawn prior to commencement of the drug infusion.

4.5.2. Drug Withholding in an Individual Subject During the 14 Day Treatment Period

Chemistries, liver function studies, CBC and differentials, and INR studies will be evaluated before drug is administered on each day that these studies are drawn as described in the attached Schedule of Assessments.

The following situations, laboratory abnormalities, or adverse events will lead to withholding of drug treatment *during the treatment course*: (Note: Day 0 is the first day of infusion)

1. Withdrawal of consent
2. Pregnancy for a female subject
3. Anaphylaxis requiring hemodynamic support (i.e., epinephrine and/or blood pressure medications) or mechanical ventilation.
4. Hepatic abnormalities*:
    a. Defined as total bilirubin >1.3 mg/dL on Day 1, ≥2.0 mg/dL on other days.
        i. The exception will be for subjects diagnosed prior to randomization with Gilbert's syndrome. These subjects will be monitored with GGT levels. They may receive drug if the total bilirubin exceeds these levels provided the GGT is ≤ 3 times the ULN. Otherwise, drug will be withheld.
    b. AST level >2 times ULN on Day 1. AST, ALT or LDH ≥3.0 times ULN on other days
5. Thrombocytopenia*: Defined as a platelet count < 140,000 on Day 1 and < 100,000 on other days.
6. Neutropenia*: Defined as <1000 cells/mm^3 (grade 3).
7. Anemia*: Defined as hemoglobin ≤ 8.5 g/dL or a drop in ≥2g/dL compared with prior to infusion to a value < 10.0 g/dL.
8. Coagulopathy*: INR > 0.1 above the upper limit of normal at the laboratory.
9. Fever: Grade 3 pyrexia on Day 0 or 1.
10. Other adverse events: Defined as a grade 3 or higher adverse event, regardless of relatedness to study drug, except for: lymphopenia, hypoglycemia, hyperglycemia,

fatigue/malaise, insomnia, cheilitis, dry skin, nail changes, hot flushes/flashes, headache, myalgia, flu-like symptoms.

11. Any medically important event such as a concurrent illness, complications or abnormal laboratory test result that, in the opinion of the investigator, contraindicates continued dosing of study drug.

A laboratory test result meeting any of the above abnormalities noted by (*) should be confirmed on the same day as the initial test. Drug dosing will not occur while awaiting confirmation of the laboratory abnormality. If laboratory abnormalities are confirmed, or if any of the other situations listed above occur, the drug infusions will be discontinued in that participant and the procedures listed below will be followed. The drug infusions may not be resumed. Depending on the severity of the event, further reporting may be required as outlined below.

If the laboratory test is not confirmed when tested on the same day, drug dosing may be continued at the discretion of the site investigator.

The Study Chair, TNCC, and Medical Monitor will be notified within 24 hours of any subject who is discontinued from study drug dosing. Participants who are discontinued from teplizumab dosing will continue to receive follow-up care and evaluation as scheduled.

4.5.3. Further Evaluation after Withholding Infusions

The following are minimal assessments to be performed for those participants in whom an infusion is withheld (as described above):

1. LIVER TESTS: Total and direct bilirubin, AST, ALT, alkaline phosphatase the day the adverse event occurs and followed with frequent laboratory studies in order to establish the day that the event resolves. Additional studies may include an abdominal ultrasound to assess liver status and GI consult when necessary. If GGT, AST or ALT is > 3 x ULN and bilirubin >2 x ULN, evaluation should be done to determine if there is a cause other than study drug for these abnormalities (e.g., acute viral hepatitis, alcoholic and autoimmune hepatitis, biliary tract disorders, cardiovascular causes such as right heart failure or concomitant medications).
2. HEMATOLOGIC TESTS: CBC, differential, INR, D-dimer, and fibrinogen the day the adverse event occurs. The peripheral blood smear will be studied for evidence of RBC fragmentation. The CBC and differential and INR, will be frequently repeated in order to establish the day that the event resolves.
3. DRUG LEVELS: Teplizumab levels
4. ADDITIONAL SAMPLES**:** Serum samples for storage and potential future analysis.

4.6. Interruption of Enrollment/Trial Cessation

Sections 4.5.2 and 4.5.3 describe monitoring and procedures for withholding drug treatment in individual patients. This section lists clinical and laboratory adverse events that will necessitate interruption of enrollment in the trial as a whole. As part of their ongoing safety review, the DSMB will make independent judgments regarding other adverse events requiring trial interruption.

1. Any drug related death*

2. Occurrence of anaphylaxis during study treatment in any participant. Anaphylaxis is defined in this protocol as a requirement of hemodynamic support or mechanical ventilation.
3. Stopping of drug infusions for criteria listed in section 4.5.2 in more than 2 of the first 10 enrolled subjects or in more than 20% of the total number of teplizumab treated subjects.
4. Grade 3 cytokine release syndrome (according to CTCAE criteria) at any time in more than 2 of the first 10 or more than 20% of the total number of teplizumab treated subjects.
5. The occurrence of ALT or AST ≥3x ULN and bilirubin >2x ULN at any time in any one subject, with the exception of those who have been diagnosed with Gilbert's syndrome prior to randomization and have met the criteria listed in section 4.5.2 for this occurrence.
6. Grade 3 hypotension at any time in more than 2 of the first 10 or more than 20% of the total number of teplizumab treated subjects.
7. Grade 3 thrombocytopenia at any time in more than 2 of the first 10 or more than 20% of the total number of teplizumab treated subjects.
8. Clinical mononucleosis syndrome which may include: Grade 2 or above fever, pharyngitis, lymphoadenopathy, splenomegaly, or rash, with detectable EBV viral load more than one week after the last dose of drug in any 3 of the first 10 or more than 25% of the total number of teplizumab treated subjects.
9. Severe adverse event: defined by CTCAE criteria of grade 3 or greater in any 2 of the first 3 patients or 3 of the first 7 drug treated patients at any time with the exception of Grade 3 lymphopenia within the first 30 days of drug treatment . In addition, severe adverse event of grade 3 or greater in more than 20% of the total number of teplizumab treated subjects.
10. Failure of the absolute number of lymphocytes to recover to 80% of the pretreatment level 2 months after the final dose of drug in 2 of the first 10 or 20% of the total number of teplizumab treated subjects.

In the event that these criteria are met, study enrollment will be suspended and the Institutional Review Boards/Ethics Committees/Research Ethics Board (IRB/EC/REB), and FDA and other applicable regulatory authorities will be notified that enrollment has been interrupted in order to perform a safety review of the enrolled subjects. The safety review will include a comprehensive evaluation of the safety experience from this trial as well as data from other ongoing studies with teplizumab in other disease settings. Before enrollment will resume, a satisfactory report of the safety review will be provided to the FDA, other applicable regulatory authorities, Institutional Review Boards/Ethics Committees/Research Ethics Boards (IRB/EC/REB), and the DSMB.

*During this trial, any death event will be temporarily considered unexpected and potentially drug-related until the event is adjudicated by the DSMB. In this event, the trial will be interrupted, including dosing of subjects already enrolled and enrollment of new subjects, until the death event is adjudicated by the DSMB and deemed "unlikely to be related to study drug."

4.7. Prophylactic Medications

Ibuprofen and antihistamine will be administered prophylactically prior to teplizumab/placebo infusion on the first 5 days of treatment. Further dosing of Ibuprofen, antihistamines, and/or acetaminophen can be used as needed for fever, malaise, headache, arthralgia, or rash.

5. STUDY VISIT ASSESSMENTS

The schedule of evaluations and laboratory studies is presented in Appendix 3, Schedule of Assessments. A summary of assessments for the Protocol is given below.

5.1. General Assessments

General assessments for this Protocol will include:

- Informed consent
- Inclusion/exclusion criteria
- Medical history including lifestyle and participant experience assessment
- Physical examination including height/weight, abdominal circumference
- Concomitant medications
- Adverse events

5.2. Laboratory Assessments

The following clinical laboratory assessments will be performed during the study as described in the Schedule of Assessments (SOA):

- Chemistry (sodium, potassium, chloride, CO2, glucose, BUN, creatinine)
- Liver function tests (ALT, AST, LDH, alkaline phosphatase, total protein, albumin, total and direct bilirubin). Prior to each infusion, GGT will be run locally in subjects with Gilbert's syndrome.
- Hematology (complete blood count with differential and platelets)
- INR
- Purified protein derivative (PPD) test
- Urine pregnancy test
- Antibodies to HIV, hepatitis B (antiHBcAb, HBsAg), hepatitis C (HCV)
- Cytomegalovirus antibodies (CMV IgG and IgM) and viral load
- Epstein-Barr virus antibodies (EBV IgG, IgM and EBNA) and viral load as indicated
- Samples for virology and other immunization titers
- ECG

5.3. Mechanistic Outcome Assessments

TrialNet will perform immune and genetic assays to further understand mechanisms that may be underlying the type 1 diabetes disease process and response to therapy. For this purpose, samples for PBMC, DNA, RNA, plasma, and serum will be obtained. HLA testing may be done either under the auspices of TrialNet Natural History/Pathway to Prevention or this protocol.

5.4. Metabolic Outcome Assessments

Metabolic assessments will consist of:

1. OGTT
   - o Primary study outcome - Glucose tolerance status. The diagnostic criteria for diabetes from the 2003 Report of the Expert Committee on the diagnosis and classification of diabetes will be used (34). This study will be performed every 6 months or more frequently if clinically indicated based on a random glucose level of $\geq$ 200 mg/dL.
   - o The C-peptide and insulin data from the OGTT will be used to measure insulin secretion.
   - o The insulin, glucose and C-peptide data from the OGTT will be used to measure insulin sensitivity.

2. HbA1c
   - o Measure of glycemic control.

5.5. Laboratory Measures Related to Teplizumab Administration

Laboratory tests to measure drug level and immune response to the drug:

- Trough drug levels of teplizumab will be measured during the last 4 days of mAb administration in 12 subjects of each of the age strata: ≥ 16 yrs, 12-15 yrs, and 8-11 yrs.
- Antibodies against teplizumab will be measured at month 3 in 12 subjects from each age strata: ≥ 16 yrs, 12-15 yrs, and 8-11 yrs.

5.6. Visit Windows

The baseline visit must occur within 7 weeks after qualifying OGTT (with the exception that individuals who are febrile at the time of the scheduled baseline visit, may have up to an additional 5 days). Visit 14 must be +/- 2 days. Visit 15 is to be +/- 4 days. All other visits described in the Schedule of Assessments can be ± 3 weeks.

6. ADVERSE EVENT REPORTING AND SAFETY MONITORING

6.1. Adverse Event Definition

6.1.1. Adverse Event

In this clinical trial, an adverse event is any occurrence or worsening of an undesirable or unintended sign, symptom or disease whether or not associated with the treatment and study procedures.

Throughout the study, the investigator must record all adverse events on source documents. Events not related to diabetes onset, hypoglycemia, or hyperglycemia which are Grade 2 or greater per the NCI CTCAE (see Section 6.1.4. Grading Event Severity below) must be reported to TNCC. The investigator should treat participants with adverse events appropriately and observe them at suitable intervals until the events resolve or stabilize.

Adverse events may be discovered through:

• observation of the participant;
• questioning the participant;
• unsolicited complaint by the participant

Questioning of the participant should be conducted in an objective manner.

6.1.2. Serious Adverse Event

A serious adverse event (SAE) or reaction is defined as "any adverse event occurring at any dose that suggests a significant hazard, contraindication, side effect, or precaution." This includes but is not limited to any of the following events:

1. Death. A death that occurs during the study or that comes to the attention of the investigator during the protocol-defined follow-up after the completion of therapy must be reported whether it is considered to be treatment related or not.
2. A life-threatening event. A life-threatening event is any adverse therapy experience that, in the view of the investigator, places the participant at immediate risk of death from the reaction as it occurred.
3. Inpatient hospitalization or prolongation of existing hospitalization.
4. Persistent or significant disability.
5. An event that required intervention to prevent permanent impairment or damage. An important medical event that may not result in death, be life threatening, or require hospitalization may be considered an SAE when, based on appropriate medical judgment, it may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.
6. Congenital anomaly or birth defect.
7. Grade 4 or higher lymphopenia for 7 or more days occurring in the first 30 days after the start of the teplizumab/placebo infusion.
8. Grade 3 or higher lymphopenia occurring anytime later than the first 30 days after the start of the teplizumab/placebo infusion.

Regardless of the relationship of the adverse event to study drug, the event must be reported as a serious adverse event if it meets any of the above definitions.

6.1.3. Unexpected Adverse Event

An adverse event is considered unexpected when the nature (specificity) or severity of the event is not consistent with the risks described in the Investigator's Brochure or the informed consent document.

6.1.4. Grading Event Severity and Causality

TrialNet has adopted usage of the National Cancer Institute (NCI) Common Technology Criteria for Adverse Events (CTCAE) and/or study-specific criteria for classification to describe the severity of adverse events. Hypoglycemia and hyperglycemia will be reported as adverse events only in the case of requiring the assistance of others due to loss of consciousness or DKA. TN Investigators will also provide an assessment of relationship of AE to study drug as not, unlikely, possibly, probably, or definitely related.

6.2. Adverse Event Reporting and Monitoring

Adverse events will be reported to the TrialNet Coordinating Center. The investigator will grade their severity according to common toxicity criteria or study-specific criteria and will make a determination of their relation to therapy. Events will be assessed and reported consistent with the ICH Guideline for Good Clinical Practice and per the guidance of the DHHS Office for Human Research Protections (OHRP).

The adverse event case report form for the protocol must be completed for all reportable adverse events (AE). For reporting serious adverse events (SAE), the TrialNet MedWatch Form should also be completed and faxed to the TNCC *within 24 hours of when the site was notified of the event.* This will be reviewed by the TrialNet Medical Monitor, the TrialNet Safety Committee, and the DSMB as appropriate. Deaths must be reported immediately. Event outcome and other follow-up information regarding the treatment and resolution of the event will be obtained and reported when available, if not known at the time the event is initially reported. The follow-up information should contain sufficient detail to allow for a complete medical assessment of the case and an independent determination of possible causality.

Adverse events will be assessed by the TrialNet Medical Monitor. The DSMB will conduct regular safety reviews approximately every three to six months (and otherwise as needed) of adverse events by treatment group assignment. Serious adverse events as well as adverse events leading to study discontinuation will be reviewed by the DSMB. All adverse events will also be reported to MacroGenics by the TNCC.

7. PARTICIPANT SAFETY

7.1. Protecting Against or Minimizing Potential Treatment Risks

Subjects will not be enrolled who have other active serious medical problems. Frequent monitoring of patients with history, physical examination, and laboratory studies will allow for early identification of adverse events. All participants will be required to have adequate hemoglobin to allow safe frequent venipuncture. Every attempt will be made to minimize the number of venipunctures.

All infusions will take place in a facility that has resuscitation capabilities.

Participants will be counseled by study personnel and requested to avoid pregnancy for 1 year following drug administration for safety purposes. This applies to females on study and female sexual partners of males.

7.1.1. Prohibited Medications

Participants will be instructed not to use Prednisone, other immunosuppressive agents, or chronic inhaled or nasal corticosteroids during this trial in order to reduce infectious risks and to prevent possible impact on progression to diabetes. However, as an intention to treat study, no individual will be withdrawn from analysis if this occurs.

Participants who receive teplizumab/placebo will be instructed not to receive live vaccinations for 1 year after dosing. In addition, participants should not receive vaccination with a killed virus vaccine less than 4 weeks after treatment with study drug unless approved by the study chair or the study ID team.

7.2. Expected Side Effects and Adverse Events

A full description of the adverse events experienced by subjects in trials using teplizumab is in the Investigator's Brochure. The descriptions below highlight the most common drug related events and potential adverse events.

7.2.1. Hematologic

The drug causes a reduction in the number of circulating lymphocytes. Grade 3 or higher lymphopenia has been seen during drug administration in 15% of subjects. However, in more than 85% of individuals, circulating lymphocytes return to ≥80% of baseline values by 2 months after initiation of treatment. A single SAE (i.e., prolonged CD4 cytopenia) has occurred in a subject who was given 2 times the proposed dose of drug. This subject did not develop infections and the CD4 cytopenia resolved spontaneously after two years.

Neutropenia, eosinophilia, and thrombocytopenia have also been seen during drug administration. Overall these adverse events have occurred in < 5% of subjects but have been up to grade 3 in < 2% of individuals. They have resolved spontaneously or with withholding of drug in all cases. This risk will be mitigated by having platelet and neutrophil count reviewed before administration of teplizumab/placebo as indicated in the SOE. No specific therapy for the eosinophila is planned. Specific treatment stopping rules are described in Section 4.5.2.

Mild anemia has been seen in 21.9% of subjects. This risk will be mitigated by having the hemoglobin reviewed before administration of teplizumab/placebo as indicated in the SOA.

7.2.2. Cytokine Release Syndrome

Cytokine release syndrome (CRS) has been described in 5.7% of drug treated subjects – the syndrome was mild or moderate in 5/6 reported subjects. Compared to FcR binding anti-CD3 antibodies like OKT3, the CRS that has occurred with Teplizumab is reduced in frequency and severity. The clinical experience to date suggests that the occurrence of CRS may be seen with the initial doses of the drug and is dose related. The single reported case of moderate disseminated intravascular coagulation was related to the occurrence of cytokine release syndrome. In a previous phase II trial (using a drug dose that is 2 times higher than the proposed dose), symptoms of CRS—including headache, nausea, vomiting, fever, myalgias, arthralgias, and shaking—occurred over the first 3 days of drug treatment, but subsequently resolved. The potential for occurrence of cytokine release syndrome has led to the drug withholding rules listed in Section 4.5.2.

Manifestations of CRS have also included hyperbilirubinemia and increased liver function tests. In the PK/safety trial (ITN017AI), a grade 4 direct hyperbilirubinemia, which may have been a manifestation of a cytokine release syndrome, was observed.

Transient increases in the alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) up to 5 times normal (grades 1 and 2) levels have been seen in all trials. These abnormalities have been transient. Grade 1 hypoalbuminemia has been seen in patients receiving the anti-CD3 mAb with other immunosuppressive agents for prevention of transplant rejection.

This risk will be mitigated by having INR and liver function tests, including bilirubin, reviewed before administration of teplizumab/placebo as described in the SOE and the specific drug withholding rules listed in Section 4.5.2.

7.2.3. Lymphoproliferative Disease

Although not raised as an issue in the single-dose studies of hOKT3γ1 (Ala-Ala), immunosuppression of any sort may predispose participants to additional risks such as infection or lymphoproliferative disease. On a theoretical basis, this risk is minimal since the total duration of immunosuppression is short. Clinical experience in transplant recipients, treated with other biologic agents, suggests that the risk of lymphoproliferative disease is highest in participants who develop infections with EBV around the time of immunosuppression. Nonetheless, the lymphoproliferative syndrome associated with reactivation of EBV infection that was seen in an islet transplant patient treated with teplizumab and other immunosuppressive drugs occurred in a subject who was EBV IgG+ before study entry. Therefore, a careful history will be taken regarding development of mononucleosis-like illnesses during the period preceding and after study enrollment. Subjects will be screened for CMV viral load. Subjects will be screened for EBV infection with serology (VCA IgG, VCA IgM, and EBNA) and EBV viral loads as indicated. The finding of a positive viral load will preclude enrollment for at least 90 days after the viral load becomes undetectable. In this situation, the subject will need to repeat OGTT assessments to ensure continued eligibility for the trial. Samples for EBV and CMV viral will be obtained after drug treatment as described in the SOE. These will be routinely measured in symptomatic subjects. This aggressive monitoring scheme will allow us to determine whether changes in lymphocyte subsets are associated with reactivation of latent viruses.

7.2.4. Anti-idiotype Responses

Anti-idiotype antibodies have been detected in up to 50% of patients administered teplizumab. The presence of these antibodies may diminish efficacy of future cycles of study drug and/or lead to manifestations of antigen-antibody complexes such as serum-sickness illness or hypersensitivity reactions. The titer of these anti-idiotype antibodies has been < 1:1000 and patients with anti-idiotype antibodies have been retreated with Teplizumab without adverse effects or detectable changes in the efficacy of the drug. To date, no adverse effects have been reported as a result of these antibodies.

7.2.5. Infection

As with any therapy that suppresses the immune system, there is a risk of developing infections. On a theoretical basis, this risk is minimal, as the total duration of immunosuppression is short. Overall, in open labeled trials with teplizumab, 49.5% of subjects have experienced infections of any kind. Of these, 48.6% were classified as mild or moderate. There have been two cases of TB reported in Protégé trial participants in India and Ukraine respectively. However, trial remains blinded so relatedness is not known at this time.

An increase in herpes virus infections has occurred with teplizumab administration. Specifically, up to 10% of subjects have had a transient increase in EBV viral load, but this was not necessarily associated with an increase in mononucleosis like syndrome. There have been case reports of herpes zoster in the Protégé study. Some occurred during dosing and others were over 270 days after commencing dosing. All subjects recovered and none of the cases were serious.

This risk will be mitigated by having subjects report even mild illness between study visits. They will be specifically asked about infectious adverse events during the study visit, and they will be monitored regularly for infections and appropriate anti-infective therapy will be instituted if indicated. Consultation with TrialNet infectious disease team will be available. All infectious adverse events will be reviewed by the TrialNet ID team, Medical Monitor and DSMB if serious.

7.2.6. Rash

Rash has been seen in 42-62% of subjects treated with drug. The rashes that have been observed include a macular rash on the face, neck, and trunk, as well as a maculopapular rash on the extremities. The latter rash has occurred on the hands and feet and has resolved spontaneously but with peeling of the skin. Biopsies of the rash performed in two subjects showed histologic findings of spongiosis consistent with eczematous dermatitis. A severe rash occurred in one subject, receiving hOKT3γ1(Ala-Ala) for prevention of islet allograft rejection on the 3rd day of drug administration. It was classified as severe and the patient was hospitalized. A biopsy of the rash showed a moderate mixed perivascular dermal infiltrate consistent with a drug reaction. Supportive care was given, the drug dosage was reduced, and the rash resolved.

7.3 Pregnancy

Pregnant and lactating women will not be included in the study. Females must have a negative pregnancy test prior to enrolling in the study and will be required to use a reliable and effective form of birth control during the study. Male participants will also be required to prevent pregnancy in their partners. At every study visit the sexual activity of participants of reproductive age will be re-assessed. If a subject who was previously sexually inactive becomes sexually active, s/he will be counseled about the need to use a reliable and effective form of birth control. Female subjects of childbearing potential will also be required to undergo urine pregnancy tests at regular intervals including prior to teplizumab/placebo administration. A positive pregnancy test will result in holding of scheduled drug infusion.

All pregnancies that are identified during the study must be followed to conclusion and the outcome of each must be reported. The investigator should be informed immediately of any pregnancy whether occurring in a female participant or the female partner of a male participant. The investigator should report all pregnancies to TrialNet within the same timeframe (24 hours) as SAEs, using the SAE report form. Monitoring of the participant should continue until the conclusion of the pregnancy, and a follow-up SAE report form detailing the outcome of the pregnancy should be submitted to TrialNet.

8. STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

Analyses of study data will be conducted to address all objectives of the trial and other interrelationships among data elements of interest to the investigators and of relevance to the objectives of the study. Analyses by gender and race/ethnicity, as appropriate, are also planned.

Primary analysis of treatment effect will be conducted under the intention-to-treat principle whereby outcome data from all subjects randomized will be included regardless of treatment compliance.

8.1 Primary Outcome

The primary outcome is the elapsed time from random treatment assignment to the development of diabetes or time of last contact among those randomized.

Criteria for diabetes onset (T1DM) are, based on glucose testing, or the presence of unequivocal hyperglycemia with acute metabolic decompensation (diabetic ketoacidosis). One of the following criteria must be met on two occasions as soon as possible but no less than one day apart for diabetes to be defined:

1. Symptoms of diabetes plus casual plasma glucose concentration $\geq$ 200 mg/dL (11.1 mmol/l). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.

2. Fasting plasma glucose ≥ 126 mg/dL (7 mmol/l). Fasting is defined as no caloric intake for at least 8 hours.

3. 2 hour plasma glucose ≥ 200 mg/dL (11.1 mmol/l). The test should be performed using a glucose load containing the equivalent of 1.75g/kg body weight to a maximum of 75 g anhydrous glucose dissolved in water.

It is preferred that at least one of the two testing occasions involve an OGTT.

Cases identified will be confirmed as having diabetes if the glucose values to make these determinations were obtained in a TrialNet laboratory as part of an OGTT. Cases diagnosed with diabetes by symptoms and casual glucose >200mg/dL or by other criteria than the above will be adjudicated by the TrialNet Diabetes Adjudication committee.

8.2 Primary Analysis

The study design is a randomized double-blind placebo controlled trial. The primary objective of the TrialNet Anti-CD3 Trial is to assess the effect of teplizumab versus control on the risk of diabetes onset in the target population as defined by the eligibility criteria.

The cumulative incidence of diabetes onset over time since randomization within each treatment group will be estimated using the Kaplan-Meier method (proportion surviving diabetes-free as a function of time). The difference between groups in the cumulative incidence functions, and the associated hazard functions, will be tested at the 0.025 level, one-sided, using the Cox Proportional Hazards (PH) model, stratified by age and the OGTT status (confirmed abnormal or not) at prior to randomization (35, 36). The estimates of cumulative incidence of diabetes and the test will be adjusted for age at enrollment. The relative risk of diabetes onset between groups will be estimated from the PH model. The critical values will be determined by the group-sequential procedure outlined in the section entitled Interim Monitoring Plan below.

8.3 Secondary Outcomes and Analyses

A variety of secondary analyses are planned, some of which will include the following.

1. Subgroup analyses will be conducted comparing the effects of teplizumab versus control on the risk of diabetes within subsets of the study cohort. Subgroups of the population will be classified by age (stratum) gender, race/ethnicity, specific antibody status at baseline, and presence or absence of HLA DQB1*0602. Differences in the treatment effect between subgroups will be tested using a covariate by treatment group interaction term in a PH model.

Similar analyses will be conducted using the values of quantitative baseline factors including weight, BMI, and the immunologic and metabolic factors described in Section 5 that include the autoantibody titers, basal C-peptide, OGTT stimulated C-peptide (peak and AUC mean), and measures of insulin resistance modeled from the OGTT. The treatment effect within the quantitative levels of each factor will be assessed through a covariate by treatment group interaction in a PH model. Such an analysis will also be conducted to assess the effects of age as quantitative covariate.

Additional covariates may be defined during the conduct of the study. The reporting of the analyses will distinguish between factors specified prior to primary analysis and those identified post-hoc during analysis.

2. Longitudinal analyses will assess the effects of teplizumab versus control treatment on immunologic and metabolic markers over time up to the onset of diabetes. Differences between groups in the mean levels of quantitative factors over time will be assessed using a normal errors linear model for repeated measures. Differences between groups in the prevalence of qualitative factors over time will be assessed using generalized estimating equations for categorical measures. Generalized estimating equations may also be employed for the analysis of quantitative factors when the normal errors assumptions are violated (37).

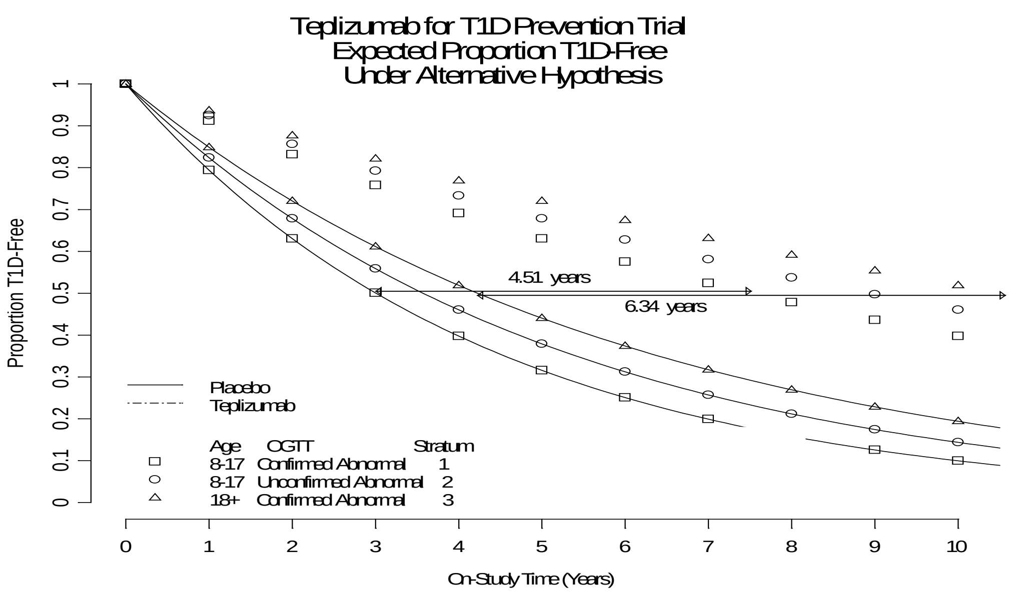
Once a subject develops diabetes, the subject will have reached the primary outcome of the study. However, the subject may still be followed for assessment of other outcomes that will permit continued longitudinal assessment of metabolic and immunologic parameters. This requirement may be satisfied through participation in another study in TrialNet.

3. The association of demographic, genetic, immunologic, metabolic, and lifestyle factors, among others, both at baseline and over time, with the risk of diabetes onset will be assessed in Cox PH Models over time. The effects of changes in longitudinal factors on diabetes risk will be assessed using time-dependent covariates for these factors. Analyses will be conducted separately within the treatment and control groups, and differences between groups in covariate effects (group by covariate interactions) will be assessed.

8.4 Study Power and Sample Size

Applying the eligibility criteria for this study to the data from the Natural History Study (TN-01), hazard and accrual rates were estimated from the TrialNet Natural History/Pathway to Prevention Study (PTP; TN-01) for the three eligible strata: 1) ages 8-17 with a confirmed abnormal OGTT, 2) ages 8-17 with an abnormal OGTT that is not confirmed, (expanded eligibility criteria), and 3) 18 or older with a confirmed abnormal OGTT. Assumptions included a constant risk over time and 25% probability of agreeing to participate. For those subjects that never received a confirmatory OGTT in the PTP study we presumed they are divided by stratum size between stratum 1 and stratum 2 in the same proportions as these groups are in the PTP Study. Likewise, hazard rates for T1DM of these grouped strata were determined by weighted average of the hazard rates from the PTP data. That is, for the 8-17 age groups the 3 strata: a) Abnormal OGTT confirmed: HR=0.1771 and accrual rate: 14.6 (35% by size) b) Abnormal but no confirmatory test: HR=0.3370 and accrual rate: 14.1 (33% by size) c) Abnormal OGTT followed by normal OGTT: HR=0.1222 and accrual rate: 13.5 (32% by size) were weighted to derive the hazard rates for strata 1 and 2. In a similar manner the accrual for 8-17 age groups was determined yielding: 22.0 and 20.3 (multiplied by 0.25, probability of agreeing to participate) for strata 1 and 2, respectively.

The estimated hazard rate is 0.231, 0.194 and 0.164 per year for strata 1, 2, and 3, respectively. The median time to T1DM onset for the control group based on a constant hazard rate is 3.01, 3.57, and 4.23 years for strata 1, 2, and 3, respectively. The effect size for this trial is a 60% reduction in the risk of T1DM (i.e., hazard ratio of experimental to control equals 0.4). This reduction in risk translates into a median time to T1DM of 7.52, 8.93, and 10.6 years for the teplizumab group for strata 1, 2, and 3, respectively (the increase is 4.51, 5.36, and 6.34 years, respectively). These design characteristics are displayed in the graph below.



The primary hypothesis test will be the Wald test of the treatment assignment variable when modeling the time to T1DM using the Cox model adjusting for baseline age and OGTT status (expanded eligibility criteria). To achieve statistical power of 80% for a one-sided Wald test at the 0.025 significance level and the effect size described above, will require enrollment and follow-up of enough participants to observe 40 subjects with T1DM onset (38) (this is the “event” sample size in contrast to the study sample size). This event sample size reflects the combination of the study sample size and the amount of follow-up at which the fixed-sample primary hypothesis test may be conducted. Although group sequential testing will be employed, the method of Lan and DeMets maintains the power while controlling the type I error used in determining the fixed sample size.

Participants <18yo will undergo an OGTT prior to the first infusion. The results of this study will be incorporated into the analysis of the primary outcome variable but will not be used to determine eligibility for the study. The study sample size and duration are variable when fixing the "event" sample size. In the absence of any safety concerns and evoking any stopping rules, closing accrual should not occur until sufficient participants are accrued so that projections (based on the observed T1DM rates and the actual accrual pattern) indicate that within a reasonable follow-up period the event sample size will be achieved. The constant hazard rate assumption is retained to compute the initial projection. The projected annual accrual is 5.5, 5.1 and 1.6 for strata 1, 2 and 3, respectively. Allowing for a 5% per year drop-out rate and the approximately 3 dozen subjects already enrolled, the study will need to accrue a total of 71 subjects in 3 years and follow all those enrolled for another 4 years beyond the last enrolled subject to achieve a statistical power of 80% (39).

Note the accrual period and the study sample size are only projections since the actual accrual rate, the control hazard rate and the loss to follow-up rate are estimates from the PTP Study or other similar trials. Furthermore, the over-all hazard rate is sensitive to the age distribution of the enrolled study population which is also an approximation. As the study progresses, projections of the study duration will be computed based on the observed data (noncomparative treatment analysis) and if in conflict, will be brought to the attention of the DSMB and the TrialNet governing body to determine the best course of action.

8.5 Interim Monitoring Plan

Interim analyses will be conducted when 50% of the expected number of cases of T1DM have been observed and will be reviewed by the TrialNet Data and Safety Monitoring Board (DSMB) for assessment of effectiveness and safety. The same primary endpoint analysis described above will be used to evaluate the evidence of a treatment group effect during interim analyses. The Wald test from the PH model will be transformed to a z-score (with negative values indicating a reduction in risk in the teplizumab treated group). If a group sequential stopping boundary is crossed, the DSMB may terminate the trial early. The Lan and DeMets (40) spending function with an O'Brien-Fleming boundary will be used to protect the type I error probability for the primary outcome analyses, and to assess the significance of the interim results periodically during the trial. The spending function (α_1) that approximates the O'Brien-Fleming boundaries is:

$$\alpha_1(t^*) = 2 - 2\Phi\left[\frac{Z_{\alpha/2}}{\sqrt{t^*}}\right]$$

where t^* is the information fraction ($0 < t^* \leq 1$) and α is the fixed-sample type I error (i.e., 0.025).

The DSMB will also be informed if there is a serious lack of evidence of a treatment effect (i.e. futility analysis). The boundaries are based on the paper by Lachin (41). The study should be stopped based on the futility of rejecting the null hypothesis at the completion of the trial if: $Z_{HR}(t^*) \geq 0$ when $0.5 \leq t^* < 0.8$ or if $Z_{HR}(t^*) \geq -0.8$ when $t^* \geq 0.8$.

Using Lachin's formulas a onetime use of either boundary for the design parameters above ($\theta \equiv Z_{1-\alpha} + Z_{1-\beta} = 2.8$) raises the type II error to approximately 0.204 and 0.202, respectively. For larger values of t^* the increase to the error probability is even less. Furthermore, by the laws of probability a single use of each rule will increase the type II error no more than the sum of the increase (i.e., 0.15+0.004+0.002 = 0.156).

Additional analysis will assess potential adverse outcomes of treatment and will assess the incidence of all severe adverse events.

8.6. Withdrawal Criteria- Individual Subjects

An intent–to-treat approach will be used. Subjects will not be replaced. All data acquired prior to termination for the reasons outlined below will be included in the primary analysis unless patient withdraws consent. Every effort will be made to conduct a final study visit with the participant and participants will be followed clinically until, if applicable, all adverse events resolve.

- Withdrawal of consent
- Withdrawal by the participant
- Withdrawal by the investigator
- Intercurrent illness or event that precludes further visits to the study site or ability to evaluate disease

9. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GOOD CLINICAL PRACTICE

9.1. Statement of Compliance

This study will be conducted in compliance with the protocol and consistent with current Good Clinical Practices (GCP), adopting the principles of the Declaration of Helsinki, and all applicable regulatory requirements (*ICH E6, 45CFR46, and FDA 21CFR sections 11, 50, 56, 312)*.

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate Independent Ethics Committee/Research Ethics Board (IEC/REB) or Institutional Review Board (IRB). Any amendments to the protocol or consent materials must also be approved before they are implemented.

9.2. Participating Centers

Participating TrialNet clinical sites must have an appropriate assurance, such as a Federal-wide Assurance (FWA) or an Unaffiliated Investigators Agreement (UIA), with the Office for Human Research Protections (OHRP), since they are actively engaged in research and provide informed consent. The protocol and consent forms will be approved by Institutional Review Boards or Ethics Committees/Research Ethics Boards at each of the participating clinical sites. HIPAA and applicable local regulations will be followed by each participating institution in accordance with each institution's requirements. The participating international sites will obtain approval from their corresponding review boards in accordance with their local procedures and institutional requirements.

The investigator is required to keep accurate records to ensure the conduct of the study is fully documented. The investigator is required to ensure that all source documentation is legibly completed for every participant entered in the trial.

The investigational sites participating in this study will maintain the highest degree of confidentiality permitted for the clinical and research information obtained from participants participating in this study. When a subject participates in this study at more than one TrialNet site, sharing of this information is required. Medical and research records will be maintained at each site in the strictest confidence. However, as a part of the quality assurance and legal responsibilities of an investigation, the investigational site must permit authorized representatives of the sponsor(s) and regulatory agencies to examine (and when required by applicable law, to copy) records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress. Unless required by the laws permitting copying of records, only the coded identity associated with documents or other participant data may be copied (obscuring any personally identifying information). Authorized representatives, as noted above, are bound to maintain the strict confidentiality of medical and research information that may be linked to identify individuals. The investigational site will normally be notified in advance of auditing visits.

9.3. Informed Consent

The process of assuring that individuals (and parent/guardian if less than 18 years of age) are making an informed decision about participating in this study includes both verbal and written communication. Written materials include a Participant Handbook and written consent forms. There are several consent forms for this study. One is a Screening consent form that describes the procedures, risks, and benefits, and eligibility requirements for the study. The second is the Intervention consent form, which describes the procedures, risks, and benefits for the remainder of the study. A third consent form is for use at clinical sites that will be performing the post-treatment visits, but not the treatment visits. The consent forms will be reviewed with participants (and their guardian in the case of participants under 18 years of age) and the participant will be given time to review the written consent form and ask questions. An assent form has also been developed for participants less than 18 years of age (unless local IRB requirements differ in procedure).

As part of the informed consent process, the participant and/or parent or guardian (if the participant is less than 18 years of age) will also be required to complete a short, written Volunteer Understanding Quiz that is designed to ensure that the subject understands the study, as well as what is being asked of him/her. The participant will be given a copy of their signed consent/assent forms.

The consent process will be conducted by qualified study personnel (the Trial or Study Coordinator and/or Investigator or other designee). All participants (or their legally acceptable representative) must read, sign and date a consent form prior to participation in the study, and/or undergoing any study-specific procedures.

The informed consent form must be updated or revised whenever there is new, clinically significant information applicable to the safety of the participants, when indicated for a protocol amendment, and/or whenever any new information becomes available that may affect a patient's participation in the study.

Subjects will be re-consented if they reach the age of 18 years while enrolled in the study.

9.4. Study Subject Confidentiality

Study records with the study subject's information for internal use at the clinical sites will be secured at the study site during the study. Identifying study subject information may be shared between TrialNet clinical sites that are involved in study procedures and/or oversight of the participant. At the end of the study, all records will continue to be kept in a secure location. There are no plans to destroy the records.

Study subject data, which is for reporting purposes, will be stored at The TrialNet Coordinating Center. Data sent to the Coordinating Center will identify participants by the unique TrialNet Identification Number. The data entry system at the Coordinating Center is a secured, password protected computer system. At the end of the study, all study databases will be archived at the Coordinating Center for long-term storage. De-identified safety data will be shared with MacroGenics during the course of the study so the company may meet its regulatory reporting requirements as the drug manufacturer and IND sponsor. Additional de-identified data will be shared with MacroGenics at the end of the study.

Stored samples including genetic samples could be utilized to learn more about causes of type 1 diabetes, its complications (such as eye, nerve, and kidney damage) and other conditions for which individuals with diabetes are at increased risk, and how to improve treatment. The results of these future analyses, and any mechanistic studies will not be made known to the participant.

9.5. Risks and Benefits

The risks of this study are presented in this protocol, the Investigator's Brochure and informed consent form. There is no guaranteed benefit to subjects for their participation in the study. This study will examine whether intervention with teplizumab will delay or prevent the onset of diabetes, but there is no guarantee that this will occur. However, all subjects will benefit from close monitoring for the development of diabetes. This close monitoring significantly reduces the morbidity typically associated with clinical onset of disease.

Special consideration regarding risks and benefits for children is described in section 2.5.

9.6. Ethics

The study protocol, along with the required informed consent forms, will be approved by each participating institution's Institutional Review Board (IRB) or Ethics Committee/Research Ethics Board (EC/REB) at international sites prior to the initiation of any research procedures (at the site). In addition to details described in the sections above (informed consent, confidentiality, and risks and benefits) the investigators have reviewed and considered ethical ramifications in the design and development of this protocol. The investigators have made every effort to minimize and monitor risks and discomforts to participants throughout the course of the study.

10. STUDY ADMINISTRATION

10.1. Organizational Structure

This study is part of Type 1 Diabetes TrialNet, which is funded by the National Institutes of Health. Funding will cover the costs of administration and laboratory tests associated with this study during the participant's period of follow-up.

10.2. Role of Industry

The IND holder is MacroGenics, Inc. MacroGenics will provide teplizumab and placebo for the study and financial support for clinical trial monitoring supplemental to standard TrialNet procedures. Eli Lilly and Company holds an exclusive license from MacroGenics to develop and commercialize teplizumab. Under TrialNet's direction, MacroGenics will perform measurements such as PK and anti-teplizumab antibodies as indicated on coded samples. Data and data analysis will be conducted by TrialNet investigators.

10.3. Groups and Committees

10.3.1. Anti-CD3 Prevention Study Chair

The Study Chair and TrialNet executive committee will receive periodic reports from the TNCC on the progress of the study. These will include accrual rates and baseline demographic characteristics. Interim data summaries provided to others (except those that could lead to unmasking of the treatment arms) will first be supplied to the Study Chair for review. Criteria and results of ongoing monitoring of the TrialNet labs in terms of reproducibility will also be provided on a routine basis and reported on during Anti-CD3 Prevention Study Committee meetings, as scheduled. As appropriate, abstracts and manuscripts dealing with the progress of the trial shall be directed by the Study Committee.

10.3.2. TrialNet Chairman's Office and TrialNet Coordinating Center

The TrialNet Chairman's Office and TNCC will work together in providing leadership to the TrialNet study group to include protocol and manual preparation, training for clinical sites, development of statistical design for each study, and analysis of study results. The TNCC will also coordinate interactions among the participating TrialNet clinical centers, test laboratories including TrialNet core laboratories and other subcontract laboratories, NIDDK, and other sponsoring agencies.

10.3.3. Clinical Sites

Each Principal Investigator at the participating TrialNet clinical site will oversee all operations at that site. The clinical sites will forward all laboratory and data collection form information to the TNCC for analysis. Conference calls and site visits, as needed, will facilitate evaluation of the trial management. Certain TrialNet sites will be involved in recruitment and follow up of subjects and some sites will also administer study drug.

10.3.4. Diabetes Adjudication Committee

A TrialNet Diabetes Onset Adjudication Committee will review all relevant information for each subject who does not meet the criteria stated in section 8.1 but has been otherwise diagnosed as having developed diabetes. The Committee will determine whether the diagnosis of diabetes in each of these subjects is sufficiently sound so as to include that subject among the cases who have reached the primary outcome in the statistical analysis. The Committee will be masked to treatment assignment as it reviews each case masked to treatment assignment.

10.3.5. Clinical Site Monitoring

In order to conduct this study with established research principles, site visits will be conducted during the study to evaluate study conduct. All sites will be monitored by the Coordinating Center and appropriate TrialNet committees for patient enrollment, compliance with protocol procedures, completeness and accuracy of data entered on the case report forms (CRFs), and the occurrence and reporting of adverse events (AEs) and serious adverse events (SAEs).

10.4. Medical Monitor and Data Safety and Monitoring Board (DSMB)

All adverse events will be recorded on the adverse event forms, which will be sent to the local IRBs, per their reporting requirements, and to the TNCC according to reporting guidelines.

An independent physician will be designated to serve as the medical monitor for this study who will maintain regular contact with the study and the study chair. (S)he will review all adverse event reports, masked to treatment assignment, and will file event reports with regulatory authorities as appropriate.

The DSMB will meet approximately every 3 months and as needed to review indicators of safety. In addition, they will meet every 6 months to review the interim effectiveness and potential toxicity of the study treatments based on interim analyses of indicators of effectiveness and safety prepared by the TNCC separately by treatment group. The DSMB will independently evaluate whether there are grounds to modify or discontinue the study.

10.5. Sample and Data Storage

Samples to be stored for research purposes will be located at the NIDDK Repository and at TrialNet Sites. While TrialNet is active, the use of the samples will be restricted to TrialNet researchers unless researchers from outside of TrialNet obtain approval from the TrialNet Steering Committee and the NIDDK to utilize the samples. Samples that are obtained for pharmacokinetics and measurement of anti-teplizumab antibodies may be made available to MacroGenics for analysis. All samples will be coded with unique study numbers, but TrialNet researchers will be able to identify samples if it is necessary to contact participants for reasons of health or for notification to them about future studies. Approval from the TrialNet Steering Committee and the NIDDK would be required before such linkage could occur. Researchers from outside of TrialNet will not be permitted to identify samples.

Data collected for this study will be sent to the TNCC. After the study is completed, the safety study data will be sent to MacroGenics by the TNCC to allow integration of all safety data on teplizumab.

De-identified data will be stored at the NIDDK Repository, under the supervision of the NIDDK/NIH, for use by researchers including those outside of TrialNet.

When TrialNet is completed, samples will continue to be stored at the NIDDK Repository Sites. Since the stored data will be fully de-identified upon the completion of TrialNet, it will no longer be possible to identify samples. Thus, whereas a sample can be destroyed upon a participant's request during the existence of the TrialNet, it can no longer be destroyed once TrialNet is completed. However, there will still be the potential to link data derived from the samples with data that had been derived from TrialNet studies. Once TrialNet is completed, researchers will only obtain access to samples through grant proposals approved by the NIDDK. The NIDDK will convene an external panel of experts to review requests for access to samples.

10.6. Preservation of the Integrity of the Study

The scientific integrity of the trial dictates that results be reported on a study-wide basis; thus, an individual Center will not report the data collected from its site alone. All presentations and publications using TrialNet trial data must protect the main objectives of the trial. Data that could be perceived as threatening the study outcome will not be presented prior to release of the primary study outcomes. Approval as to the timing of presentations of data and the meetings at which they might be presented will be granted by the TrialNet Steering Committee. Study results should be discussed with the news media only upon authorization of the Steering Committee, and never before the results are presented. Any written statements about this study that are shared with national media must be approved by TrialNet before release.

10.7. Participant Reimbursement and Compensation

Participants will be compensated for each visit attended in the study.

APPENDIX 1: Natural History/Pathway to Prevention to Teplizumab in At-Risk Relatives Study Flow Chart for Subjects ≥18yo

Natural History Screening

Procedures	First or second degree relative Initial AutoAntibody draw
Results to move on	**AutoAntibodies (AA)** At least one autoantibody confirmed positive, or two autoantibodies present

Natural History Risk Assessment

Procedures	Confirmation of autoantibody status, OGTT, HLA
Results to move onto teplizumab	**AutoAntibodies (AA)**[1] At least two confirmed diabetes related autoantibodies confirmed to be present on two occasions. Confirmation of 2 positive autoantibodies must occur within six months prior to randomization, but the confirmation does not have to involve the same 2 autoantibodies. **OGTT** Fasting Plasma Glucose ≥ 110 mg/dL and < 126 mg/dL OR 2-hr Plasma Glucose ≥140 mg/dL and < 200 mg/dL OR 30, 60, or 90 minute glucose ≥ 200 mg/dL

Teplizumab eligibility visit

Procedures	Screening consent is signed. OGTT[2] (unless abnormal glucose tolerance has been previously confirmed from a test in TrialNet within 7 weeks (52 days) of randomization), laboratory assessments, PPD, History, PE, Volunteer Quiz, Education.
Results to move on	OGTT with abnormal glucose tolerance (if applicable) Does not meet any exclusion criteria[3]

Teplizumab Baseline and Randomization visit

Procedures	Intervention consent signed. Baseline laboratory assessments, dosing of teplizumab/placebo

[1]If autoantibodies are not confirmed positive on the second test a tiebreaker draw will be required.
[2]If the OGTT confirms abnormal glucose tolerance, the subject is eligible to proceed with randomization. If the OGTT is consistent with diabetes, the subject is not eligible for enrollment. He/She may be eligible for enrollment in the future if subsequent studies do not confirm the diagnosis of diabetes and the above entry criteria are met. If neither abnormal glucose tolerance or diabetes is confirmed, the subjects may have repeat studies as outlined above to meet the entry criteria.
[3]If subject not eligible or unwilling to participate in teplizumab in at-risk, subject may be followed in TN Natural History/Pathway to Prevention Study.

APPENDIX 2: Natural History/Pathway to Prevention to Teplizumab in At-Risk Relatives Study Flow Chart for Subjects <18yo

Natural History Screening

Procedures	First or second degree relative Initial AutoAntibody draw
Results to move on	**AutoAntibodies (AA)** At least one autoantibody confirmed positive, or two autoantibodies present

⇩

Natural History Risk Assessment

Procedures	Confirmation of autoantibody status, OGTT, HLA
Results to move onto teplizumab	**AutoAntibodies (AA)[1]** At least two confirmed diabetes related autoantibodies confirmed to be present on two occasions. Confirmation of 2 positive autoantibodies must occur within six months prior to randomization, but the confirmation does not have to involve the same 2 autoantibodies. **OGTT** Fasting Plasma Glucose $\geq$ 110 mg/dL and < 126 mg/dL OR 2-hr Plasma Glucose $\geq$140 mg/dL and < 200 mg/dL OR 30, 60, or 90 minute glucose ≥ 200 mg/dL

⇩

Teplizumab eligibility visit

Procedures	Screening consent is signed. Random Glucose[2] or OGTT if not previously performed, laboratory assessments, PPD, History, PE, Volunteer Quiz, Education.
Results to move on	Non-Fasting Random Glucose <200mg/dL, or OGTT with abnormal glucose tolerance Does not meet any exclusion criteria[3]

⇩

Teplizumab Baseline and Randomization visit

Procedures	Intervention consent signed. Baseline laboratory assessments, OGTT, dosing of teplizumab/placebo

[1]*If autoantibodies are not confirmed positive on the second test a tiebreaker draw will be required.*
[2]*If the Random Glucose is consistent with diabetes, the subject is not eligible for enrollment. He/She may be eligible for enrollment in the future if subsequent studies do not confirm the diagnosis of diabetes and the above entry criteria are met. If neither abnormal glucose tolerance or diabetes is confirmed, the subjects may have repeat studies as outlined above to meet the entry criteria.*
[3]*If subject not eligible or unwilling to participate in teplizumab in at-risk, subject may be followed in TN Natural History/Pathway to Prevention Study.*

APPENDIX 3 - Schedule of Assessments

Week of Trial															2		6													
Month of Trial																		3	6	12	18	24	30	36	42	48	54	60	66	72[10]
Day of Trial	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	20														
Visit number	-1	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Study drug[1]		X	X	X	X	X	X	X	X	X	X	X	X	X	X															
Chemistries [2,3]	X	X	X	X	X	X	X	X					X		X		X		X	X		X		X		X		X	X	X
INR[2]	X	X	X	X	X	X	X						X		X															
CBC with diff[2]	X	X	X	X	X	X	X	X		X			X		X	X	X	X	X	X	X	X		X		X		X	X	X
Liver Function[2,3]	X	X	X	X	X	X	X	X					X		X		X		X	X		X		X		X		X	X	X
mAb levels[4]		X										X	X	X	X															
Anti-teplizumab response	X																	X												
EBV and CMV viral loads[5]	X															X	X	X		X		X		X		X				
EBV/CMV serology	X																			X		X		X		X				
History/Physical exam[6]	X	X					X					X			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Oral Glucose Tolerance[7]	X	X																X	X	X	X	X	X	X	X	X	X	X	X	X
HIV, HepB and C serology	X																													
PPD test	X																													
Urine pregnancy test	X	X																X	X	X	X	X	X	X	X	X	X	X	X	X
HbA1c	X																	X	X	X	X	X	X	X	X	X	X	X	X	X
Mechanistic assessments[8]		X														X		X	X	X	X	X	X	X	X	X	X	X	X	X
glycemic status[9]	X																													
EKG		X																		X										

1= Drug dosing: Day 0: 51 mcg/m2, Day 1: 103 mcg/m2, Day 2: 207 ug/m2, Day 3: 413 ug/m2, Days 4 - 13: 826 ug/m2.
2= These studies must be reviewed prior to drug administration (see protocol re: drug withholding). INR and CBC with diff are done locally at all specified visits.
3= Liver Function tests (ALT, AST, LDH, alkaline phosphatase, total protein, albumin, total and direct bilirubin) and Chemistries (sodium, potassium, chloride, CO2, glucose, BUN, creatine) are done locally at specified visits 0-13. Tests are done centrally at all other specified visits.
4= These PK samples will be done on the first 12 subjects in each of the age strata: ≥ 16, 12-15, and 8-11.
5= At Screen, EBV viral load will be measured on all subjects except those VCA IgG+, VCA IgM-, EBNA+ by serology. After study drug treatment, EBV and CMV viral load samples will be collected from all subjects. Viral loads will be measured in symptomatic subjects.
6= Directed/limited physical exam for visits at month 3, 6, 18, 30, 42, 54, 66, and then q 6 months.
7= Screening OGTT only applicable for participants ≥18yo, unless abnormal glucose tolerance has been previously confirmed by a test in TrialNet within 7 weeks (52 days) of randomization. If OGTT consistent with DM, repeat within 1 month. Glucose, insulin, and C-peptide are collected at each OGTT. Participants <18yo will undergo an OGTT at or prior to the baseline visit (visit 0). The results of this study will not be used to determine eligibility for the study
8=Includes samples for RNA, plasma, serum, DNA, measures of B and T cell number and function to understand the effect of therapy on the immune system and infectious disease.
The schedule for these assessments may vary as appropriate. At no time will the blood draw volume exceed what is allowable according to the subject's age and body weight (For subjects <18, 5ml/kg per visit, 9.5 mg/kg in an 8 week period.)
9= Screening random glucose only applicable for participants <18yo not undergoing an OGTT on the same day. All subjects will have interim contact with study personnel for formal inquiry about adverse events, presence or absence of blurred vision, polyuria, polydypsia, unintended weight loss. In addition, random glucose samples will be obtained at 3 month intervals in which there is no OGTT scheduled. Those with symptoms or glucose ≥200mg/dl will undergo fasting glucose or OGTT evaluation.
Additional samples will be drawn in the case of drug withholding (see protocol: drug withholding 4.5.2)

10= The assessments at month 72 will be repeated every 6 months.

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Summary of TN10 Protocol Amendments

1. **Protocol Amendment V. 22June2010:**
    a. Addition of language to include Research Ethics Board (REB) responsibilities, pursuant to international sites.

2. **Protocol Amendment V. 25June2012**
    a. Removed section 3.9.1 Staggered Enrollment.

3. **Protocol Amendment V. 17September2012**
    a. Standardized T1DM acronym throughout protocol for consistency.
    b. Section 2.3.4 – Inclusion of updated data on AEs and results from the ABATE trial.
    c. Section 2.3.5 – Inclusion of updated information on study results from DELAY trial.
    d. Section 2.3.6 – Inclusion of updated information on study results from Protégé trial.
    e. Section 2.3.7 – Inclusion of updated information on study completion of the Protégé Encore trial.
    f. Section 3.3.1 – revised age criteria to provide clarification of enrollment of subjects from TN01 who were < 45 years of age, but now may be > 45 years old.
    g. Section 3.3.1 – Addition of ZnT8 autoantibodies as one of two Abs required for eligibility.
    h. Section 3.3.1 – added statement that T1D clinical history of proband may be reviewed by the eligibility committee to determine study eligibility.
    i. Section 5.2 – added IgM and EBNA to reflect current monitoring procedures for possible infections.
    j. Section 7.2.1 – added eosinophilia as a possible side effect of drug therapy to be consistent with new Investigator's Brochure (12/22/2011).
    k. Section 7.2.5 – added wording regarding herpes virus infections to be consistent with new Investigator's Brochure (12/22/2011).
    l. Section 8.4 – Modified to reflect revisions to the enrollment period, study duration, and sample size.
    m. Appendix 2: Schedule of Assessments – minor wording changes and inclusion of revised EBV monitoring procedures; revision of footnotes to reflect inclusion of revised EBV monitoring procedures.

4. **Protocol Amendment V. 25June2014**
    a. Section 3.3.1 – modified to reflect revisions to study inclusion criteria related to OGTT requirements. Individuals < 18 years of age must have one abnormal OGTT prior to enrollment, and those 18 years and older must have two consecutive abnormal OGTTs.
    b. Section 3.3.2 – modified to reflect revisions to study exclusion criteria mentioned above; Addition of exclusion criteria for AST or ALT > 1.5 ULN; addition of language to allow those with Gilbert's syndrome, in the absence of other causes to hyperbilirubinemia.
    c. Section .3.9.1 – modified to provide additional clarification on sample size, accrual period, and study duration revisions.
    d. Section 4.2 – modified to include further clarification of initial visit procedures related to OGTT criteria mentioned in point a.
    e. Section 4.3 - modified to include further clarification of initial visit procedures related to OGTT criteria mentioned in point a.

f. Section 4.5.1 – modified to provide further clarification of drug administration and dosage calculations.
g. Section 4.5.2 – modified to provide further clarification for drug withholding criteria in subjects with Gilbert's syndrome.
h. Section 4.5.3 – modified to provide further evaluation after withholding infusions for subjects with Gilbert's syndrome.
i. Section 4.6 – modified to provide information related to Gilbert's syndrome and trial cessation.
j. Section 5.2 – Addition of working related to requirement of liver function testing for subjects with Gilbert's syndrome.
k. Section 7.3 – modified to provide clarification related to pregnancy test requirements, including language that these are only applicable to females of childbearing potential.
l. Section 8.3 – modified to provide clarification related to secondary outcomes and analyses.
m. Section 8.4 – modified to reflect revisions to the study power and sample size.
n. Section 8.5 – modified to reflect revisions to the interim monitoring plan.
o. Section 9.4 – modified to reflect current data sharing, storage, and security procedures.
p. Appendix 2 – revised to reflect modification to entry criteria for subjects < 18 years old, related to removal of requirement for confirmed abnormal OGTT.

Statistical Analysis Plan

TRIAL FULL TITLE	**ANTI-CD3 MAB (TEPLIZUMAB) FOR PREVENTION OF DIABETES IN RELATIVES AT-RISK FOR TYPE 1 DIABETES MELLITUS (Protocol TN-10)**
EUDRACT NUMBER	
Phase	Phase IIb
SAP VERSION	
ISRCTN NUMBER	
SAP VERSION DATE	October 26, 2017
TRIAL STATISTICIAN	Brian Bundy
TRIAL CHIEF INVESTIGATOR	Kevan Herold
SAP AUTHOR	Jeffrey Krischer

Table of Contents

1. Abbreviations and Definitions

AE	Adverse Event
ADA	American Diabetes Association
AUC	Area Under the Curve
BMI	Body Mass Index
DSMB	Data Safety Monitoring Committee
ITT	Intent to Treat
OGTT	Oral Glucose Tolerance Test
PH	Proportional hazards
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
T1DM	Type 1 Diabetes Melitis

2. Introduction

Preface

The rationale for this study is that individuals with immunologic markers of T1DM and abnormal glucose tolerance are at very high risk for progression to overt disease. They have a condition that differs from overt diabetes only in the duration of the autoimmune process that results in beta cell destruction. It is hypothesized that intervention at the “prediabetic” stage is likely to be more effective than intervention in those in whom frank hyperglycemia has developed and beta cell function has deteriorated further because insulin production is greater before compared to after the diagnosis.

Purpose of the analyses

Analyses of study data will be conducted to address all objectives and other interrelationships among elements of study data of interest to the investigators and of relevance to the objectives of the study. Analyses by sex, age, and race/ethnicity, as appropriate, are also planned.
All primary analyses will be conducted under the intention-to-treat principle whereby all outcome data in all randomized subjects will be included, regardless of treatment compliance.

Primary Outcome

The primary outcome is the elapsed time from random treatment assignment to the development of diabetes or time of last contact among those enrolled and determined to be eligible.

Criteria for diabetes onset are, as defined by the American Diabetes Association (ADA), based on glucose testing, or the presence of unequivocal hyperglycemia with acute metabolic decompensation (diabetic ketoacidosis)(1,2). For criteria based on glucose testing, <u>one of the following criteria must be met</u> on two occasions as soon as possible but no less than one day apart for diabetes to be defined:

1. Symptoms of diabetes plus casual plasma glucose concentration $\geq$ 200 mg/dL

(11.1 mmol/l). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.

2. Fasting plasma glucose ≥ 126 mg/dL (7 mmol/l). Fasting is defined as no caloric intake for at least 8 hours.

3. 2 hour plasma glucose ≥ 200 mg/dL (11.1 mmol/l) on an Oral Glucose Tolerance Test (OGTT). The test should be performed using a glucose load containing the equivalent of 1.75g/kg body weight to a maximum of 75 g anhydrous glucose dissolved in water.

3. Primary Analysis

The study design is a randomized double-blind placebo controlled trial. The primary objective of the TrialNet Anti-CD3 Trial is to assess the effect of teplizumab versus control on the risk of diabetes onset in the target population as defined by the eligibility criteria.

The cumulative incidence of diabetes onset over time since randomization within each treatment group will be estimated using the Kaplan-Meier method (proportion surviving diabetes-free as a function of time). The difference between groups in the cumulative incidence functions, and the associated hazard functions, will be tested at the 0.025 level, one-sided, using the Cox Proportional Hazards (PH) model with discrete time intervals at the 6 month OGTT intervals (3,4). The estimates of cumulative incidence of diabetes and the test will be adjusted for age at enrollment as a continuous covariate. As well, age will also be tested using the 8-17 and 18-45 year old groups reflecting the planning parameters of this study (Section 5, below). The relative risk of diabetes onset between treatment arms will be estimated from the PH model. The critical values will be determined by the group-sequential procedure outlined in the section entitled Interim Monitoring Plan below.

Using a step-up procedure additional covariates will be tested and included in the model only if they improve the log-likelihood at 0.05 level (2-sided). This will be accomplished with the treatment assignment variable included but the inclusion/exclusion of the candidate covariate will be completely independent of the treatment variable's impact on the model. The candidate covariates to be tested for inclusion include, but are not limited to: sex, BMI, HbA1c, HLA, baseline AUC C-peptide, baseline OGTT (fasting, 2-hour, AUC), autoantibody types and titers (mIAA, GADA, IA2A). The Wald test associated with treatment variable in the full, adjusted model will be used for the test of treatment effect described in the previous paragraph. Thus, the adjustment of the significance level is superfluous.

For the 2 subjects who failed to get a baseline OGTT, their first OGTT on study will be used instead. The analysis will consider an OGTT in the diabetic range at baseline (yes or no) as a covariate as described above.

4. Secondary Outcomes and Analyses

A variety of secondary analyses are planned, some of which will include the following.

1. For those quantitative baseline factors (including weight, BMI, and the immunologic and metabolic factors, including the autoantibody titers, basal C-peptide, OGTT

stimulated C-peptide (peak and AUC mean), and measures of insulin resistance modeled from the OGTT) entered into the model used in the primary analysis, an attempt will be made to distinguish levels or intervals that correspond to different diabetes risk. The treatment effect within the quantitative levels of each factor will then be assessed through a covariate by treatment group interaction in a PH model. Such an analysis will also be conducted to assess the effects of age as quantitative covariate as described above.

2. Additional exploratory subgroup analyses will be conducted comparing the effects of teplizumab versus control on the risk of diabetes within subsets of the study cohort. Pre-specified subgroups of the enrolled population will be classified by age (stratum) sex, race/ethnicity, autoantibody type and titer at baseline, OGTT in the diabetic range at baseline (yes/no), and HLA risk categories, including the presence or absence of HLA DQB1*0602. Differences in the treatment effect between subgroups will be tested using a covariate by treatment group interaction term in a PH model (3).

3. Although there is limited power, the diabetes-free proportion at 1,2 and 3 years after randomization will be calculated and compared using the Kaplan-Meier estimates of the proportions and the Greenwood estimates of the variance.

4. Longitudinal analyses will assess the effects of teplizumab versus control treatment on immunologic and metabolic markers over time up to the onset of diabetes. Differences between groups in the mean levels of quantitative factors over time will be assessed using a normal errors linear model for repeated measures. Differences between groups in the prevalence of qualitative factors over time will be assessed using generalized estimating equations for categorical measures. Generalized estimating equations may also be employed for the analysis of quantitative factors when the normal errors assumptions are violated (5).

5. Once a subject develops diabetes, the subject will have reached the primary outcome of the study. However, the subject may still be followed for assessment of other outcomes that will permit continued longitudinal assessment of metabolic and immunologic parameters. This requirement may be satisfied through participation in another study in TrialNet.

6. The association of demographic, genetic, immunologic, metabolic, and lifestyle factors, among others, both at baseline and over time, with the risk of diabetes onset will be assessed in Cox PH Models over time. The effects of changes in longitudinal factors on diabetes risk will be assessed using time-dependent covariates for these factors. Analyses will be conducted separately within the treatment and control groups, and differences between groups in covariate effects (group by covariate interactions) will be assessed. Assumptions regarding a constant hazard rate will be tested to examine differential efficacy over time.

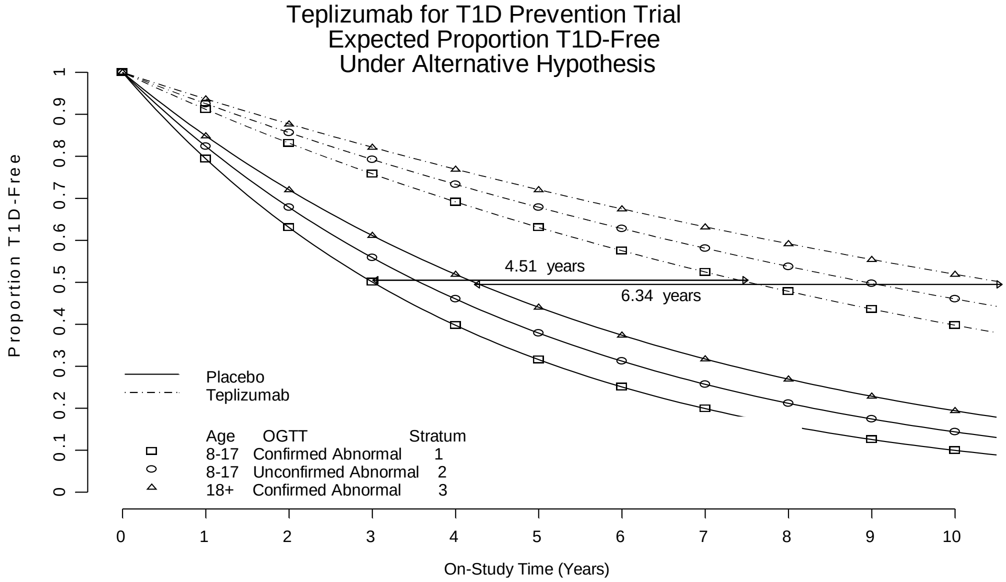
7. Evidence that the HR is not constant over the period of follow up will be assessed and the risk of diabetes will be assessed in a PH model. The primary test for treatment effect is based on the Cox model which assumes a proportional hazards between treatment groups. If there is a true treatment effect but it is not proportional over the follow-up period the test will have substantially less statistical power than stated in the statistical section of the protocol. Therefore, to establish a reasonable

guide to pursue the possibility of a non-proportional treatment effect we will use the guideline requiring a significance level of 0.10 or less of the Wald test from the standard Cox model. Due to the modest size of this trial, nominal level for significance when testing for violations in proportional hazards will not be specified. Rather, graphical diagnostics using Schoenfeld residuals will be employed to explore evidence for a monotonically decreasing effect of treatment over the follow-up period. We will characterize any such decreasing effect of treatment by model parameterization with monotonically deceasing benefit over the follow-up time. Initially, a treatment interaction with log-transformed time-on-study will be fit to the data and the log likelihood improvement in the fit noted. Other time transforms will be explored only to characterize mathematically the rate of the diminishing effect. Of particular interest will be characterizing the point in follow-up where the hazard ratio is 1. Any variations in proportional hazards other than a monotonically decreasing effect will not be of interest because of the possibility that it is simply random error.

5. Study Power and Sample Size

Applying the eligibility criteria for this study to the data from the Natural History Study (TN-01), hazard and accrual rates were estimated from the TrialNet Natural History/Pathway to Prevention Study (PTP; TN-01) for the three eligible strata: 1) ages 8-17 with a confirmed abnormal OGTT, 2) ages 8-17 with an abnormal OGTT that is not confirmed, (expanded eligibility criteria), and 3) 18 or older with a confirmed abnormal OGTT. Assumptions included a constant risk over time and 25% probability of agreeing to participate. For those subjects that never received a confirmatory OGTT in the PTP study we presumed they are divided by stratum size between stratum 1 and stratum 2 in the same proportions as these groups are in the PTP Study. Likewise, hazard rates for T1DM of these grouped strata were determined by weighted average of the hazard rates from the PTP data. That is, for the 8-17 age groups the 3 strata: a) Abnormal OGTT confirmed: HR=0.1771 and accrual rate: 14.6 (35% by size) b) Abnormal but no confirmatory test: HR=0.3370 and accrual rate: 14.1 (33% by size) c) Abnormal OGTT followed by normal OGTT: HR=0.1222 and accrual rate: 13.5 (32% by size) were weighted to derive the hazard rates for strata 1 and 2. In a similar manner the accrual for 8-17 age groups was determined yielding: 22.0 and 20.3 (multiplied by 0.25, probability of agreeing to participate) for strata 1 and 2, respectively.

The estimated hazard rate is 0.231, 0.194 and 0.164 per year for strata 1, 2, and 3, respectively. The median time to T1DM onset for the control group based on a constant hazard rate is 3.01, 3.57, and 4.23 years for strata 1, 2, and 3, respectively. The effect size for this trial is a 60% reduction in the risk of T1DM (i.e., hazard ratio of experimental to control equals 0.4). This reduction in risk translates into a median time to T1DM of 7.52, 8.93, and 10.6 years for the teplizumab group for strata 1, 2, and 3, respectively (the increase is 4.51, 5.36, and 6.34 years, respectively). These design characteristics are displayed in the graph below.



The primary hypothesis test will be the Wald test of the treatment assignment variable when modeling the time to T1DM using the Cox model adjusting for baseline age and OGTT status (expanded eligibility criteria). To achieve statistical power of 80% for a one-sided Wald test at the 0.025 significance level and the effect size described above, will require enrollment and follow-up of enough participants to observe 40 subjects with T1DM onset (38) (this is the “event” sample size in contrast to the study sample size). This event sample size reflects the combination of the study sample size and the amount of follow-up at which the fixed-sample primary hypothesis test may be conducted. Although group sequential testing will be employed, the method of Lan and DeMets maintains the power while controlling the type I error used in determining the fixed sample size.

Participants <18yo will undergo an OGTT prior to the first infusion. The results of this OGTT will be incorporated into the analysis of the primary outcome variable but will not be used to determine eligibility for the study. The study sample size and duration are variable when fixing the “event” sample size. In the absence of any safety concerns and evoking any stopping rules, closing accrual should not occur until sufficient participants are accrued so that projections (based on the observed T1DM rates and the actual accrual pattern) indicate that within a reasonable follow-up period the event sample size will be achieved. The constant hazard rate assumption is retained to compute the initial projection. The projected annual accrual is 5.5, 5.1 and 1.6 for strata 1, 2 and 3, respectively. Allowing for a 5% per year drop-out rate and the approximately 3 dozen subjects already enrolled, the study will need to accrue a total of 71 subjects in 3 years and follow all those enrolled for another 4 years beyond the last enrolled subject to achieve a statistical power of 80% (39).

Note the accrual period and the study sample size are only projections since the actual accrual rate, the control hazard rate and the loss to follow-up rate are estimates from the PTP Study or other similar trials. Furthermore, the over-all hazard rate is sensitive to the age distribution of the enrolled study population which is also an approximation. As the study progresses, projections of the study duration will be computed based on the observed data (noncomparative treatment analysis) and if in conflict, will be brought to the attention of the DSMB and the TrialNet governing body to determine the best course of action.

6. Interim Monitoring Plan

Interim analyses will be conducted periodically during the study and will be reviewed by the TrialNet Data and Safety Monitoring Board (DSMB) for assessment of effectiveness and safety; the TrialNet DSMB meets at least every six months to review study progress and safety. An independent medical monitor will closely monitor the events in the trial as described in section 10.4. If a group sequential stopping boundary is crossed, the DSMB may terminate enrollment into the trial early. The Lan-DeMets (7) spending function with an O'Brien-Fleming boundary will be used to protect the type I error probability for the primary outcome analyses, and to assess the significance of the interim results periodically during the trial. The spending function that approximates the O'Brien-Fleming boundaries is:

$$\alpha_1(t^*) = 2 - 2\Phi\left[\frac{Z_{\alpha/2}}{\sqrt{t^*}}\right]$$

where t^* is the information fraction ($0 < t^* \leq 1$) and α is the fixed-sample type I error (i.e., 0.025).

The DSMB will also be informed if there is a serious lack of evidence of a treatment effect (i.e. futility analysis). The boundaries are based on the paper by Lachin (41). The study should be stopped based on the futility of rejecting the null hypothesis at the completion of the trial if: $Z_{HR}(t^*) \geq 0$ when $0.5 \leq t^* < 0.8$ or if $Z_{HR}(t^*) \geq -0.8$ when $t^* \geq 0.8$.

Using Lachin's formulas a onetime use of either boundary for the design parameters above ($\theta \equiv Z_{1-\alpha} + Z_{1-\beta} = 2.8$) raises the type II error to approximately 0.204 and 0.202, respectively. For larger values of t^* the increase to the error probability is even less. Furthermore, by the laws of probability a single use of each rule will increase the type II error no more than the sum of the increase (i.e., 0.15+0.004+0.002 = 0.156).

Additional analysis will assess potential adverse outcomes of treatment and will assess the incidence of all severe adverse events.

7. General Considerations

1. Analysis Populations

The Intention to Treat Population (ITT)

The intention to treat population comprises all randomized (as planned) subjects.

Full Analysis Population

The Full Analysis Set (FAS) will comprise all subjects who received any study drug and who participated in at least one post-baseline assessment. These will be analyzed as randomized. FAS will be the primary efficacy population. So, FAS is a subset of ITT.

Per Protocol Population

The Per Protocol Set (PPS) will comprise all subjects who did not substantially deviate from the protocol as to be determined on a per-subject basis before data base lock and unblinding. These will be analyzed according to actual treatment received and stratum.

Safety Population

All subjects who received any study treatment (including control) but excluding subjects who drop out prior to receiving any treatment.

During accrual, 2 subjects enrolled on the >= 18 year of age stratum did not have a second OGTT prior to randomization to confirm abnormal glucose tolerance. Eligibility is technically unknown without the results of the missing test. Given that the these subjects had an OGTT on study indicated abnormal glucose tolerance, not necessarily consecutive, we considered these conditions sufficient to retain these subjects for analysis.

2. Timing of Analyses

The final analysis will come after sufficient numbers of events (40 or more) have been reported in the FAS population to achieve the planned 80% statistical power for the primary analysis.

3. Adjustment of Confidence Intervals and p-values

There was 1 interim analysis conducted when 18 subjects were diagnosed with T1D. We applied the Lan-DeMets decision rule as outlined in the protocol and spent type I error of p = 0.00083, one-sided. Therefore, the final analysis should be conducted at 0.0242 level (to preserve type I error). Given this almost negligible difference from 0.025, all p-values will be reported as if a fixed-sample size test was conducted and only noting the adjusted critical value p-value in the results section if the primary hypothesis test falls within the narrow range (i.e., 0.0242 – 0.025).

8. Safety Analyses

Safety will be evaluated with summary of adverse events for the safety population.
The following parameters will be assessed during the study:

Adverse Events

The summary statistics will be produced in accordance with Section 8.
Treatment emergent adverse events (AEs) are those events that occur after the baseline assessment. Only Grade 2 or greater adverse events were reported in this study. The incidence of the following AEs will be reported:

A tabular summary of AE will present: Number of subjects with any AE; Number of SAEs with outcome death; Number subjects with SAE; Number subjects with AEs leading to discontinuation of study drug, even if by protocol; Number of subjects with AEs leading to discontinuation of study; Total number of AEs; Total number of SAEs [TABLE].

The Adverse Events summary tables will include number of adverse events, the number of subjects in each treatment group in whom the event occurred, and the incidence of occurrence and should be grouped by system organ class, preferred terms and/or other interested variables (e.g., relatedness, intensity and seriousness). [TABLE]

When calculating the incidence of adverse events, or any sub-classification thereof by treatment, time period, severity, etc., each subject will only be counted once and any repetitions of adverse events will be ignored; the denominator will be the total population size.
Deaths, Serious Adverse Events and other Significant Adverse Events

All formal testing of adverse effects will be based on the subject as the experimental unit. Thus for comparing incidence of AE within system organ by treatment group, a one-sided Fisher's exact test will be conducted at 0.05 level (higher incidence in experimentally treated group is the alternative hypothesis). Also, highest AE grade will be determined for each subject and compared by treatment group using a 2 sample Wilcoxon test (one-sided at 0.05). No correction for multiple testing will be employed in order that the statistical power is maintained.

9. Reporting Conventions

P-values ≥0.01 will be reported to 2 decimal places; p-values less than 0.01 and >0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001". The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures.

10. Per Protocol Analysis

Quantifying the evidence of any dose response relationship is part of a complete analysis of any well run and completed clinical trial. This is especially true when the trial's primary outcome is negative to explore whether there is evidence that deviations from the treatment protocol may have played a role in the negative outcome. Given this we plan to assess the treatment hazard ratio by the degree of compliance to the protocol scheduled dose in a quantitative manner. Using the Cox model we will assess the evidence for an effect of treatment compliance including the entire cohort. The number of courses of treatment and/or the treatment dose will be introduced into the model to determine its effect on risk. If there is evidence that it is predictive (<=0.05, one-sided) then a treatment-courses of therapy, or dose, interaction term will be introduced to see if there is a different compliance gradient between the two treatment groups. The procedure for including covariates, such as age, will follow the set up procedure as described above under Primary and Secondary analyses.

11. Technical Details

The analysis will be performed in R, S-Plus or SAS.

The distributional assumptions as well as other assumptions underpinning the planned analyses will be checked. Final decisions regarding analysis methods and choice of explanatory variables will be taken then.

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Statistical Analysis Plan

TRIAL FULL TITLE	**ANTI-CD3 MAB (TEPLIZUMAB) FOR PREVENTION OF DIABETES IN RELATIVES AT-RISK FOR TYPE 1 DIABETES MELLITUS (Protocol TN-10)**
EUDRACT NUMBER	
Phase	Phase IIb
SAP VERSION	
ISRCTN NUMBER	
SAP VERSION DATE	March 27, 2018
TRIAL STATISTICIAN	Brian Bundy
TRIAL CHIEF INVESTIGATOR	Kevan Herold
SAP AUTHOR	Jeffrey Krischer

Table of Contents

1. Abbreviations and Definitions

AE	Adverse Event
ADA	American Diabetes Association
AUC	Area Under the Curve
BMI	Body Mass Index
DSMB	Data Safety Monitoring Committee
ITT	Intent to Treat
OGTT	Oral Glucose Tolerance Test
PH	Proportional hazards
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
T1DM	Type 1 Diabetes Mellitus

2. Introduction

Preface

The rationale for this study is that individuals with immunologic markers of T1DM and abnormal glucose tolerance are at very high risk for progression to overt disease. They have a condition that differs from overt diabetes only in the duration of the autoimmune process that results in beta cell destruction. It is hypothesized that intervention at the “prediabetic” stage is likely to be more effective than intervention in those in whom frank hyperglycemia has developed and beta cell function has deteriorated further because insulin production is greater before compared to after the diagnosis.

Purpose of the analyses

Analyses of study data will be conducted to address all objectives and other interrelationships among elements of study data of interest to the investigators and of relevance to the objectives of the study. Analyses by sex, age, and race/ethnicity are also planned.
All primary analyses will be conducted under the intention-to-treat principle whereby all outcome data in all randomized subjects will be included, regardless of treatment compliance.

Primary Outcome

The primary outcome is the elapsed time from random treatment assignment to the development of diabetes or time of last contact in the Intention to Treat Population

Criteria for diabetes onset are, as defined by the American Diabetes Association (ADA), based on glucose testing, or the presence of unequivocal hyperglycemia with acute metabolic decompensation (diabetic ketoacidosis)(1,2). For criteria based on glucose testing, <u>one of the following criteria must be met</u> on two occasions as soon as possible but no less than one day apart for diabetes to be defined:

1. Symptoms of diabetes plus casual plasma glucose concentration $\geq$ 200 mg/dL

(11.1 mmol/l). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.

2. Fasting plasma glucose ≥ 126 mg/dL (7 mmol/l). Fasting is defined as no caloric intake for at least 8 hours.

3. 2 hour plasma glucose ≥ 200 mg/dL (11.1 mmol/l) on an Oral Glucose Tolerance Test (OGTT). The test should be performed using a glucose load containing the equivalent of 1.75g/kg body weight to a maximum of 75 g anhydrous glucose dissolved in water.

3. Primary Analysis

The study design is a randomized double-blind placebo controlled trial. The primary objective of the TrialNet Anti-CD3 Trial is to assess the effect of teplizumab versus control on the risk of diabetes onset in the intention-to-treat population.

The cumulative incidence of diabetes onset over time since randomization within each treatment group will be estimated using the Kaplan-Meier method (proportion surviving diabetes-free as a function of time). The difference between groups in the cumulative incidence curves, and the associated hazard functions, will be tested at the 0.025 level, one-sided, using the Cox Proportional Hazards (PH) model with discrete time intervals at the 6 month OGTT intervals (3,4). The hazard ratio of diabetes onset between treatment arms will be estimated from the PH model. The critical values will be determined by the group-sequential procedure outlined in the section entitled Interim Monitoring Plan below. The primary test of treatment effect will be adjusted for the design strata and any covariates identified using the procedure outlined below.

Using a step-up procedure additional covariates will be tested and included in the model only if they improve the log-likelihood at 0.10 level (2-sided). This will be accomplished with the treatment assignment variable included but the inclusion/exclusion of the candidate covariates will be completely independent of the treatment variable's impact on the model. The candidate covariates to be tested for inclusion are: sex, BMI, HbA1c, HLA (DR3/4 vs. others), baseline C-peptide (fasting, peak, AUC), baseline OGTT glucose (fasting, 2-hour, AUC), autoantibody presence (mIAA, GADA, IA2A, ZnT8) at study entry. The Wald test associated with treatment variable in the full, adjusted model will be used for the test of treatment effect described in the previous paragraph. Thus, the adjustment of the significance level, as with multiple testing, is unnecessary.

4. Secondary Outcomes and Analyses

The original design of this study anticipated a two-year enrollment period and follow-up of 3 years after the last subject was enrolled for a total of 5 years. To deal with the potential loss of drug effect, the treatment arms will be compared at 5 years, as if the study did follow the original plan (i.e., had the study progressed according to the original plan with the minimum accrual, 50% of the subjects would have been followed for 4 years and 50% for 5 years. For purposes of this secondary outcome, study subjects will be censored at 5 years following their randomization date). This will be the principal pre-specified secondary objective All other planned secondary objective will be considered exploratory.

Exploratory, pre-specified secondary analyses are identified below.

1. For those quantitative baseline factors (including weight, BMI, and the immunologic and metabolic factors, including the autoantibody titers, basal C-peptide, OGTT stimulated C-peptide (peak and AUC mean), and measures of insulin secretion and insulin resistance modeled from the OGTT) entered into the model used in the primary analysis, an attempt will be made to distinguish levels or intervals that correspond to different diabetes risk. The treatment effect within the quantitative levels of each factor will then be assessed through a covariate by treatment group interaction in a PH model. Such an analysis will also be conducted to assess the effects of age as quantitative covariate as described above.

2. Additional exploratory subgroup analyses will be conducted comparing the effects of teplizumab versus control on the risk of diabetes within subsets of the study cohort. Pre-specified subgroups of the enrolled population will be classified by age (8-17 and 18-45) sex, race/ethnicity, autoantibody type and titer at baseline, OGTT in the diabetic range at baseline (yes/no), and HLA risk categories, including the presence or absence of HLA DQB1*0602 and other factors deemed important (such as site and temporal effects) Differences in the treatment effect between subgroups will be tested using a covariate by treatment group interaction term in a PH model (3) and significance levels reported regardless of whether they achieve nominal significance due to the lack of statistical power.

3. Although there is limited power, the diabetes-free proportion at 1,2 and 3 years after randomization will be calculated and compared using the Kaplan-Meier estimates of the proportions and the Greenwood estimates of the variance.

4. Longitudinal analyses will assess the effects of teplizumab versus control treatment on immunologic and metabolic markers over time up to the onset of diabetes. Differences between groups in the mean levels of quantitative factors over time will be assessed using a normal errors linear model for repeated measures. Differences between groups in the prevalence of qualitative factors over time will be assessed using generalized estimating equations for categorical measures. Generalized estimating equations may also be employed for the analysis of quantitative factors when the normal errors assumptions are violated (5).

5. Once a subject develops diabetes, the subject will have reached the primary outcome of the study. However, the subject may still be followed for assessment of other outcomes that will permit continued longitudinal assessment of metabolic and immunologic parameters. This requirement may be satisfied through participation in another study in TrialNet.

6. The association of demographic, genetic, immunologic, metabolic, and lifestyle factors, among others, both at baseline and over time, with the risk of diabetes onset will be assessed in Cox PH Models over time. The effects of changes in longitudinal factors on diabetes risk will be assessed using time-dependent covariates for these factors. Analyses will be conducted separately within the treatment and control groups, and differences between groups in covariate effects (group by covariate interactions) will be assessed. Assumptions regarding a constant hazard rate will be tested to examine differential efficacy over time.

7. Evidence that the HR is not constant over the period of follow up will be assessed and the risk of diabetes will be assessed in a PH model. The primary test for treatment effect is based on the Cox model, which assumes a proportional hazards between treatment groups. If there is a true treatment effect but it is not proportional over the follow-up period the test will have substantially less statistical power than stated in the statistical section of the protocol. Therefore, to establish a reasonable guide to pursue the possibility of a non-proportional treatment effect we will use the guideline requiring a significance level of 0.10 or less of the Wald test from the standard Cox model. Due to the modest size of this trial, nominal level for significance when testing for violations in proportional hazards will not be specified. Rather, graphical diagnostics using Schoenfeld residuals will be employed to explore evidence for a monotonically decreasing effect of treatment over the follow-up period. Also, plotting the Kaplan-Meier time-to-T1D rates by treatment group on the log-log scale will be used to quantify any diminishing effect of treatment (equal distance separation of curves indicates proportional hazard). We will characterize any such decreasing effect of treatment by model parameterization with monotonically deceasing benefit over the follow-up time. Initially, a treatment interaction with log-transformed time-on-study will be fit to the data and the log likelihood improvement in the fit noted. Other time transforms will be explored only to characterize mathematically the rate of the diminishing effect. Of particular interest will be characterizing the point in follow-up where the hazard ratio is 1. Any variations in proportional hazards other than a monotonically decreasing effect will not be of interest because of the possibility that it is simply random error.

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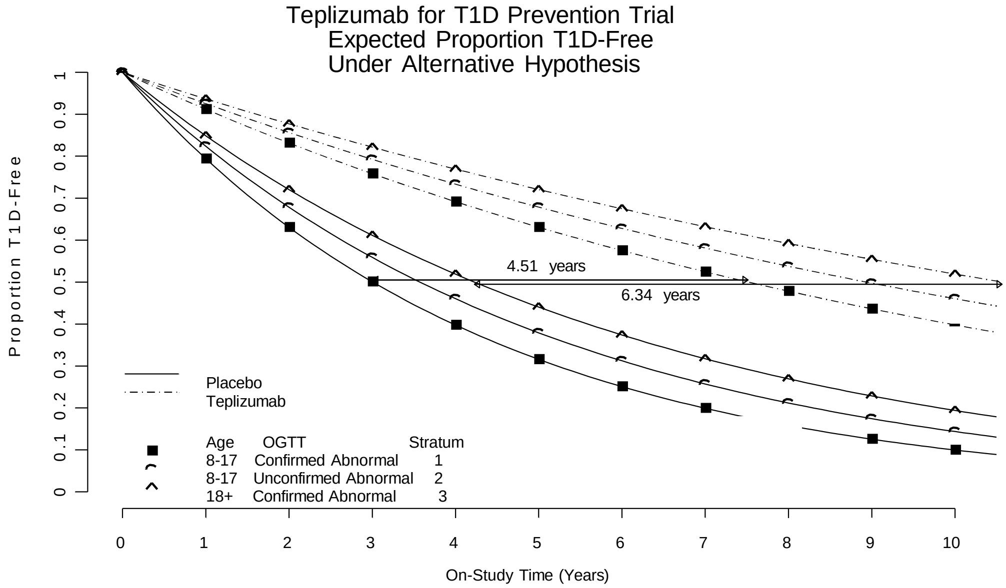

The primary hypothesis test will be the Wald test of the treatment assignment variable when modeling the time to T1DM using the Cox model adjusting for baseline age and OGTT status (expanded eligibility criteria). To achieve statistical power of 80% for a one-sided Wald test at the 0.025 significance level and the effect size described above, will require enrollment and follow-up of enough participants to observe 40 subjects with T1DM onset (38) (this is the “event” sample size in contrast to the study sample size). This event sample size reflects the combination of the study sample size and the amount of follow-up at which the fixed-sample primary hypothesis test may be conducted. Although group sequential testing will be employed, the method of Lan and DeMets maintains the power while controlling the type I error used in determining the fixed sample size.

Participants <18yo will undergo an OGTT prior to the first infusion. The results of this OGTT will be incorporated into the analysis of the primary outcome variable but will not be used to determine eligibility for the study. The study sample size and duration are variable when fixing the “event” sample size. In the absence of any safety concerns and evoking any stopping rules, closing accrual should not occur until sufficient participants are accrued so that projections (based on the observed T1DM rates and the actual accrual pattern) indicate that within a reasonable follow-up period the event sample size will be achieved. The constant hazard rate assumption is retained to compute the initial projection. The projected annual accrual is 5.5, 5.1 and 1.6 for strata 1, 2 and 3, respectively. Allowing for a 5% per year drop-out rate and the approximately 3 dozen subjects already enrolled, the study will need to accrue a total of 71 subjects in 3 years and follow all those enrolled for another 4 years beyond the last enrolled subject to achieve a statistical power of 80% (39).

Note the accrual period and the study sample size are only projections since the actual accrual rate, the control hazard rate and the loss to follow-up rate are estimates from the PTP Study or other similar trials. Furthermore, the over-all hazard rate is sensitive to the age distribution of the enrolled study population, which is also an approximation. As the study progresses, projections of the study duration will be computed based on the observed data (noncomparative treatment analysis) and if in conflict, will be brought to the attention of the DSMB and the TrialNet governing body to determine the best course of action.

6. Interim Monitoring Plan

Interim analyses will be conducted periodically during the study and will be reviewed by the TrialNet Data and Safety Monitoring Board (DSMB) for assessment of effectiveness and safety; the TrialNet DSMB meets at least every six months to review study progress and safety. An independent medical monitor will closely monitor the events in the trial as described in section 10.4. If a group sequential stopping boundary is crossed, the DSMB may terminate enrollment into the trial early. The Lan-DeMets (7) spending function with an O'Brien-Fleming boundary will be used to protect the type I error probability for the primary outcome analyses, and to assess the significance of the interim results periodically during the trial. The spending function that approximates the O'Brien-Fleming boundaries is:

$$\alpha_1(t^*) = 2 - 2\Phi\left[\frac{Z_{\alpha/2}}{\sqrt{t^*}}\right]$$

where t^* is the information fraction ($0 < t^* \leq 1$) and α is the fixed-sample type I error (i.e., 0.025).

The DSMB will also be informed if there is a serious lack of evidence of a treatment effect (i.e. futility analysis). The boundaries are based on the paper by Lachin (41). The study should be stopped based on the futility of rejecting the null hypothesis at the completion of the trial if: $Z_{HR}(t^*) \geq 0$ when $0.5 \leq t^* < 0.8$ or if $Z_{HR}(t^*) \geq -0.8$ when $t^* \geq 0.8$.

Using Lachin's formulas a onetime use of either boundary for the design parameters above ($\theta \equiv Z_{1-\alpha} + Z_{1-\beta} = 2.8$) raises the type II error to approximately 0.204 and 0.202, respectively. For larger values of t^* the increase to the error probability is even less. Furthermore, by the laws of probability a single use of each rule will increase the type II error no more than the sum of the increase (i.e., 0.15+0.004+0.002 = 0.156).

Additional analysis will assess potential adverse outcomes of treatment and will assess the incidence of all severe adverse events.

7. General Considerations

1. Analysis Populations

The Intention to Treat Population (ITT)

The intention to treat population comprises all randomized (as planned) subjects.

Full Analysis Population

The Full Analysis Set (FAS) will comprise all subjects who received any study drug and who participated in at least one post-baseline assessment. These will be analyzed as randomized. FAS will be the primary efficacy population. So, FAS is a subset of ITT.

Per Protocol Population

The Per Protocol Set (PPS) will comprise all subjects who did not substantially deviate from the protocol as to be determined on a per-subject basis before data base lock and unblinding.

Safety Population

All subjects who received any study treatment (including control) but excluding subjects who drop out prior to receiving any treatment.

During accrual, 2 subjects enrolled on the >= 18 year of age stratum did not have a second OGTT prior to randomization to confirm abnormal glucose tolerance. Eligibility is technically unknown without the results of the missing test. Given that the these subjects had an OGTT on study indicated abnormal glucose tolerance, not necessarily consecutive, we considered these conditions sufficient to retain these subjects for analysis.

2. Timing of Analyses

The final analysis will come after sufficient numbers of events (40 or more) have been reported in the FAS population to achieve the planned 80% statistical power for the primary analysis.

3. Adjustment of Confidence Intervals and p-values

There was 1 interim analysis conducted when 18 subjects were diagnosed with T1D. We applied the Lan-DeMets decision rule as outlined in the protocol and spent type I error of p = 0.00083, one-sided. Therefore, the final analysis should be conducted at 0.0242 level (to preserve type I error). Given this almost negligible difference from 0.025, all p-values will be reported as if a fixed-sample size test was conducted and only noting the adjusted critical value p-value in the results section if the primary hypothesis test falls within the narrow range (i.e., 0.0242 – 0.025).

4. Missing Data

In general, missing values will be assumed to be *missing completely at random (MCAR)* unless empirical evidence to the contrary can be established internal to the study. The methodology employed in analyzing time-to-T1D utilizes whatever follow-up has been recorded for each subject (i.e., maximum utilization of follow-up time). Presuming no evidence against MCAR, and the modest size of the trial, no methods will be employed to impute additional follow-up of subjects that drop out (i.e., lost to follow-up). All secondary endpoints will use the complete-case analysis approach which limits the analytical cohort to those subjects that have the secondary endpoint of interest measured and recorded. In modeling to adjust for risk factors associated with the endpoint (i.e. covariates), missing values will be assigned the mean from the known covariate cohort. This simple rule will be employed only if the percent missing is less than 10% for the analytical cohort. If the missing is 10% to 20% a separate indicator for missing will be included in the modeling. If the missing in exceeds 20% the covariate will be removed from consideration.

8. Safety Analyses

Safety will be evaluated with summary of adverse events for the safety population. The following parameters will be assessed during the study:

Adverse Events
The summary statistics will be produced in accordance with Section 8.
Treatment emergent adverse events (AEs) are those events that occur after the baseline assessment. Only Grade 2 or greater adverse events were reported in this study. The incidence of the following AEs will be reported:

A tabular summary of AE will present: Number of subjects with any AE; Number of SAEs with outcome death; Number subjects with SAE; Number subjects with AEs leading to discontinuation of study drug, even if by protocol; Number of subjects with AEs leading to discontinuation of study; Total number of AEs; Total number of SAEs [TABLE].

The Adverse Events summary tables will include number of adverse events, the number of subjects in each treatment group in whom the event occurred, and the incidence of occurrence and should be grouped by system organ class, preferred terms and/or other interested variables (e.g., relatedness, intensity and seriousness). [TABLE]

When calculating the incidence of adverse events, or any sub-classification thereof by treatment, time period, severity, etc., each subject will only be counted once and any repetitions of adverse events will be ignored; the denominator will be the total population size.
Deaths, Serious Adverse Events and other Significant Adverse Events

All formal testing of adverse effects will be based on the subject as the experimental unit. Thus for comparing incidence of AE within system organ by treatment group, a one-sided Fisher's exact test will be conducted at 0.05 level (higher incidence in experimentally treated group is the alternative hypothesis). Also, highest AE grade will be determined for each subject and compared by treatment group using a 2 sample Wilcoxon test (one-sided at 0.05). No correction for multiple testing will be employed in order that the statistical power is maintained.

9. Reporting Conventions

P-values ≥0.01 will be reported to 2 decimal places; p-values less than 0.01 and >0.001 will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001". The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures.

10. Per Protocol Analysis

Quantifying the evidence of any dose response relationship is part of a complete analysis of any well run and completed clinical trial. This is especially true when the trial's primary outcome is negative to explore whether there is evidence that deviations from the treatment protocol may have played a role in the negative outcome. Given this we plan to assess the treatment hazard ratio by the degree of compliance to the protocol scheduled dose in a quantitative manner.

Using the Cox model we will assess the evidence for an effect of treatment compliance including the entire cohort. The number of infusions of treatment and/or the treatment dose will be introduced into the model to determine its effect on risk. If there is evidence that it is predictive (<=0.05, one-sided) then a treatment-infusions of therapy, or dose, interaction term will be introduced to see if there is a different compliance gradient between the two treatment groups. The procedure for including covariates, such as age, will follow the set up procedure as described above under Primary and Secondary analyses.

11. Technical Details

The analysis will be performed in R, S-Plus or SAS.

The distributional assumptions as well as other assumptions underpinning the planned analyses will be checked. Final decisions regarding analysis methods and choice of explanatory variables will be taken then.

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- **Section 2: Introduction:**
    - Clarification that the primary outcome will be conducted in the Intention to Treat Population.
- **Section 3: Primary Analysis:**
    - Clarification that the primary outcome will be conducted in the Intention to Treat Population.
    - Addition of the statement: "The primary test of treatment effect will be adjusted for the design strata and any covariates identified using the procedure outlined below."
    - Changed log-likelihood from 0.05 to a 0.10 level (2-sided).
    - Clarification and expansion of covariates, including HLA DR3/4 vs. others; fasting, peak and AUC c-peptide; and the addition of the ZnT8 autoantibody.
- **Section 4: Secondary Outcomes and Analyses:**
    - Addition of a statement outlining the plan to address potential loss of drug effect by comparing the treatment arms at 5 years, as in the original study plan.
    - Addition of insulin secretion to preplanned exploratory analysis.
    - Clarification of age classifications to be utilized, and expansion to other factors deemed important such as site and temporal effects.
    - Addition of a statement that significance levels will be reported regardless of whether they achieve nominal significance, due to the lack of statistical power.
    - Addition of a statement that plotting the Kaplan-Meier time-to-T1D rates by treatment group on the log-log scale will be used to quantify any diminishing effect of treatment (equal distance separation of curves indicates proportional hazard).
- **Section 7: General Considerations:**
    - Addition of a section related to the handling of Missing Data.
- **Section 10: Per Protocol Analysis:**
    - Correction of the term "courses" to "infusions".

EDITORIAL

Traveling down the Long Road to Type 1 Diabetes Mellitus Prevention

Clifford J. Rosen, M.D., and Julie R. Ingelfinger, M.D.

Type 1 diabetes mellitus, a chronic autoimmune disease that usually begins in childhood, affects more than 1.25 million Americans,[1] and its worldwide prevalence is increasing. Although insulin was discovered almost a century ago and the technologies for administering and monitoring insulin treatment have improved quality of life and reduced complications from the condition, the disease remains incurable. Accordingly, investigative efforts have centered on prevention, aiming to either delay or prevent disease onset. However, that requires the elucidation of the relevant pathophysiological mechanisms that lead to the pancreatic beta-cell destruction thought to be the root cause of type 1 diabetes. Early work focused on the identification of infectious or toxic causes of this destruction. Then, in an article published 40 years ago in the *Journal*, Eisenbarth and colleagues suggested an alternative idea — that there was a functional interaction of HLAs with autoantibodies to insulin in patients with polyglandular autoimmune failure and type 1 diabetes.[2] These observations seeded the field of endocrine immunology and led to revisions of theories about the pathogenesis of type 1 diabetes.

Currently, the pathogenesis of the condition is thought to be due to environmental triggers that initiate autoimmune destruction of pancreatic beta cells in persons who are at genetic risk, in whom endogenous antigens are expressed on target cells and presented by a complex with class I HLA. The highest-risk HLA genotype is DR3-DQ2, DR4-DQ8 (DQ8 represents DQA1*0301-DQB1*0302 and has been found to be associated with type 1 diabetes); by 12 years of age, a child who inherits the same DR3-DQ2, DR4-DQ8 genotype as a sibling with type 1 diabetes has a greater than 75% risk of the development of autoantibodies and a greater than 50% risk of the development of diabetes.[3]

As type 1 diabetes develops, lymphocytes, particularly CD8+ T cells, infiltrate and slowly kill beta cells.[4] The onset of the disease is gradual, and three clinical stages can be defined. Stage 1 is asymptomatic, characterized purely by the presence of autoantibodies; stage 2 is defined by an impaired metabolic response to a glucose load, although other metabolic indexes, such as the glycated hemoglobin level, remain normal; and stage 3 is marked by overt insulin deficiency, hyperglycemia, and loss of beta-cell function.

Herold et al. now report in the *Journal* the results of a phase 2, randomized, placebo-controlled, double-blind trial in which teplizumab, an Fc receptor–nonbinding antibody to CD3, was evaluated in relatives of patients with type 1 diabetes.[5] Teplizumab reduces the actions of CD8+ T lymphocytes on targets such as beta cells.[6] Previous, shorter trials of teplizumab in type 1 diabetes, all involving patients with early stage 3 disease, showed promise — although in one trial the results for the composite primary outcome (i.e., the use of insulin and glycated hemoglobin) were not significant.[7-9] In contrast, the present multinational trial was planned as a prevention trial involving high-risk persons (stage 2), with the primary outcome of time to diagnosis of overt type 1 diabetes. Eligible participants had two or more diabetes-related autoantibodies and evidence of dysglycemia on oral glucose-tolerance testing. Participants were assigned in a 1:1 ratio to receive a 14-day outpatient course of intravenous teplizumab or placebo; most were children

(<18 years of age) and were followed for more than 3 years.

The results of this trial are striking, with several caveats. The annualized rates of new-onset type 1 diabetes were 14.9% per year in the teplizumab group and 35.9% per year in the placebo group. The median time to diagnosis of type 1 diabetes was 48.4 months in the teplizumab group and 24.4 months in the placebo group (hazard ratio, 0.41; 95% confidence interval, 0.22 to 0.78), after adjustment for age and antibody status. Not surprisingly, the greatest preventive benefit occurred in the first year of the trial, and the adverse-event profile showed a depression in total lymphocyte counts in the teplizumab group, although all these participants had a rebound in lymphocyte count during continued follow-up. In subgroup analyses, the presence of HLA-DR4 and the absence of HLA-DR3 were associated with more robust responses to teplizumab, as was the presence of anti–zinc transporter 8 antibodies.

Although the trial showed a marked delay in the onset of overt diabetes, the results should not be taken to imply that immune modulation constitutes a potential curative approach. Rather, these data provide strong albeit indirect evidence about the pathogenesis of beta-cell destruction and the potential to modify the course of type 1 diabetes with newer biologic agents. This trial will probably prompt the development of more refined screening criteria for treatment of persons at highest risk, although challenges in using immune modulators for type 1 diabetes remain.[10] This trial was small (76 participants) and involved only one 2-week treatment course. The duration and frequency of treatments, the long-term side effects of those therapies, the identification of subgroups of persons who do not have a response to the treatment, and the clinical course of persons who initially do have a response still need to be determined. Nevertheless, we can finally say, 40 years after Eisenbarth, that there has been substantial progress in modulating the early course of type 1 diabetes.

Disclosure forms provided by the authors are available with the full text of this editorial at NEJM.org.

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